GLYCO XX

20th International Symposium on Glycoconjugates

Abstract

November 29 – December 4, 2009
San Juan, PR, USA

Glycoconjugate Journal

Subscription Information

Glycoconjugate Journal is published nine times a year by Springer Science+Business Media, LLC (Springer), Volume 26 (9 issues) will be published in 2009.

ISSN: 0282-0080 (print version)
ISSN: 1573-4986 (electronic version)
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Printed in The Netherlands on acid-free paper.

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Periodicals postage paid at New York, New York and additional mailing offices.
Postmaster: Send address changes to: *Glycoconjugate Journal*, Springer, 233 Spring Street, New York, NY 10013, USA.

Invitation

The organizing committee of the 20th International Symposium of Glycoconjugates on behalf of the International Glycoconjugate Organization invites you to attend the biennial event to be held in San Juan, PR. GLYCO XX will be held at the Caribe Hilton Hotel, a historic waterfront resort. The hotel is just 15 minutes from the Luis Muñoz Marin International Airport, perfectly located between Condado and Old San Juan. While planning a very exciting meeting the organizing committee has also paid attention to your comfort as well as to your accompanied guests. The scientific sessions will run from early morning to the late afternoon each day and the poster session will be held in a more casual atmosphere in the evenings.

Scientific Secretariat

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History

International Glycoconjugate Organization (IGO) is affiliated with the International Union of Biochemistry and Molecular Biology and has evolved slowly since 1964. The organization has representatives from 24 countries and is supported by an Executive Committee. The main objective of the IGO is to further international collaboration for the study of glycoconjugates. One of the mechanisms the IGO uses to promote its vision is by organizing the biennial International Glycoconjugate Symposia. Therefore, the symposia are organized in different countries. The first symposium was held in Swampscott, USA by late Professor Roger Jeanloz. Following a gap of nine years the symposium was resumed in 1973 and has continued since then. Following are the dates, places and their organizers:

GLYCO	I	(1964, Swampscott, USA; late Professor Roger Jeanloz)
GLYCO	II	(1973, Lille, France; Professor Jean Montreuil)
GLYCO	III	(1975, Brighton, UK; Professor Colin Hughes)
GLYCO	IV	(1977, Woods Hole, USA; Professors John Gregory and Lennart Roden Co-chaired the meeting)
GLYCO	V	(1979, Kiel, West Germany; Professor Roland Schauer)
GLYCO	VI	(1981, Tokyo, Japan; Professor Tamio Yamakawa)
GLYCO	VII	(1983, Ronneby, Sweden; by late Professor Bengt Lindberg)
GLYCO	VIII	(1985, Houston, USA; Professor William J. Lennarz)
GLYCO	IX	(1987, Lille, France; Professor Jean Montreuil)
GLYCO	X	(1989, Jerusalem, Israel; Professor Nathan Sharon)
GLYCO	XI	(1991, Toronto, Canada; Professor Harry Schachter)
GLYCO	XII	(1993, Kraków, Poland; Professor Jerzy Koscielak)
GLYCO	XIII	(1995, Seattle, USA; Professor Sen-itiroh Hakomori)
GLYCO	XIV	(1997, Zurich, Switzerland; Professor Jürgen Roth)
GLYCO	XV	(1999, Tokyo, Japan; Professor Akira Kobata)
GLYCO	XVI	(2001, Utrecht, The Netherlands; Professor Hans Vliegenthart)
GLYCO	XVII	(2003, Bangalore, India; Professor Avadesha Surolia)
GLYCO	XVIII	(2005, Florence, Italy; Professor Guido Tettamanti)
GLYCO	XIX	(2007, Cairns, Australia; Professor Paul Gleeson)

Future IGO Meetings

GLYCO	XXI	(2011, Vienna, Austia; Professor Leo März)
GLYCO	XXII	(2013, Shanghai, China; Professor J. Gu)

Travel Information

Hotel
The Caribe Hilton Hotel will be the host hotel for Glyco XX. To get the guaranteed room rates we advise to make the room reservation early. **Reservation Code : SYMA09vation** The room rates

Travel
San Juan is well connected from all major cities in the US mainland and international flights. The airport code for Luis Muñoz Marin International Airport is SJU

American Airlines is the official airline for the GLYCO XX meeting. To receive the discounted rates use the code: **A01N9AA**.

Immigration requirements
No visa is required for the US passport holders traveling to San Juan. International Travelers, please contact the American Consulate in your country regarding visa regulations for travel to the United States. Obtaining a visa may take several weeks or months, depending on your location, so we encourage you to contact the Consulate as soon as possible. Information is also available on the U.S. Department of State website at www.travel.state.gov.

Language
The official language of the meeting is English

Local Climate
Average temperature in San Juan all year round is 82°F or 28°C.

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Glycoconj J (2009) 26:763–898
DOI 10.1007/s10719-009-9256-7

20th International Symposium on Glycoconjugates

Glycans: From Molecules to Structures to Therapeutics

November 29–December 4, 2009

Caribe Hilton Hotel, San Juan, Puerto Rico. USA

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Scientific Program

Sunday, November 29, 2009

2:00 pm–6:30 pm **Registration**

3:00 pm–4:00 pm **Opening Ceremony**
Location: **San Geronimo Ball Room - A, B & C**

Special Scientific Session: What we have Learned about Glycans and What Lies Ahead
Sunday, 4:00 pm–7:00 pm

Location: **San Geronimo Ball Room - A, B & C**
Chair: **Dipak K. Banerjee,** University of Puerto Rico, USA

1 From milk oligosaccharides to N-linked sugar chains: Our forty years journey in Glycobiology. **A. Kobata**. The Noguchi Institute, Japan.

2 The importance of unambiguous glycan structures. **Johannes F.G. Vliegenthart**. Bijvoet Center Biomolecular Research, Utrecht University, Utrecht, The Netherlands.

3 Studies on Enzymatic Formation and Degradation of Glycoproteins. **William J. Lennarz**. Department of Biochemistry and Cell Biology, State University of New York at Stony Brook, Stony Brook, New York, USA.

4 Protein Glycosylation, conserved from Yeast to Man. **Widmar Tanner.** University of Regensburg, Regensburg, Germany.

5 The past and new challenges of sialic acid research. **Roland Schauer.** University of Kiel, Institute of Biochemistry, Kiel, Germany.

6 Use of Glycomics to Target Therapeutic Enzymes. **Roscoe O. Brady.** NINDS, National Institutes of Health, Bethesda, MD, USA.

7:00 pm–8:30 pm **Welcome Reception –** ***Beach Area***

Monday, November 30, 2009

6:30 am–8:25 am **Breakfast –** ***San Cristobal Ball Room***

8:30 am–6:30 pm **Registration**

Sunrise Session I: Deciphering the Glycome: Challenges and the Rewards
Monday, 8:30 am–9:45 am

Location: **San Geronimo Ball Room - A, B & C**
Chair: **Akira Kobata,** The Noguchi Institute, Japan.

8:30 am–8:50 am 7 Mass spectrometric strategies for glycomics and glycoproteomics. **Anne Dell.** Imperial College London, London, UK.

8:55 am–9:15 am 8 Identifying and exploiting the cis and trans ligands of CD22.
James C. Paulson, Weihsu (Claire) Chen, Gladys Completo, Corwin Nycholat, Mary E. O'Reilly, Cory Rillahan, Ramya T. N. C., Hua Tian, Ying Zeng. Department of Chemical Physiology, The Scripps Research Institute, La Jolla, CA, USA.

9:20 am–9:40 am 9 Development of Glyco-cancer biomarkers using novel technologies.
Hisashi Narimatsu. Research Center for Medical Glycoscience, National Institute of Advanced Industrial Science and Technology (AIST), Tsukuba, Japan

Plenary Lecture I
Monday, 9:45 am–10:30 am

Location: **San Geronimo Ball Room - A, B & C**
Chair: **Ten Feizi,** Imperial College London, UK

10 Paulin M Rudd
Dublin-Oxford Glycobiology Laboratory, Conway Institute, University College Dublin, Belfield, Dublin, Ireland
Glycomics analysis combined with a genome-wide association study identifies loci involved in regulation of the human plasma N-glycome.

10:30 am–10:50 am **Coffee/Tea Break –** ***Foyer***

Concurrent Session 1: Glycoimmunology
Monday, 10:50 am–12:55 pm

Location: **San Geronimo Ball Room – B**
Co-Chairs: **James C. Paulson,** Scrip Research Institute, USA
Anthony J. Day, University of Manchester, UK

10:50 am–11:10 am 11 Glycans to modulate Dendritic cell mediated immune responses. **Yvette van Kooyk, Ingegorg Streng-Ouwehand, Satwinder K. Singh, Manja Litjens, Wendy Unger.** VUmc, Amsterdam, the Netherlands.

11:10 am–11:30 am 12 The regulation of immunological tolerance and autoimmunity by 9-O-acetylation of sialic acid. **Shiv Pillai, Ira Surolia, Stephan Pirnie, Annaiah Cariappa, Jesse Moya, Ajit Varki, Peter Gregersen, Kendra Taylor, Amy McQuay, Bruce Sands.** Massachusetts General Hospital, Harvard Medical School, Boston, MA, USA.

11:30 am–11:50 am 13 9-O-acetylated sialoglycoproteins in visceral leishmaniasis: The multifaceted trigger modulating the erythrocyte biology. **Angana Ghoshal[1], Sajal Samanta[1], Bibhuti Saha[2], Saulius Jarmalavicius[3], Peter Walden[3], Chitra Mandal[1].** [1]Infectious Disease and Immunology Division, Indian Institute of Chemical Biology, Kolkata, India; [2]Department of Tropical Medicine, School of Tropical Medicine, Kolkata, India; [3]Department of Dermatology, Charité-Universitätsmedizin Berlin, Humboldt University, Berlin, Germany.

11:50 am–12:10 pm 14 Galectins in innate immunity: dual functions of host soluble β-galactoside binding lectins as damage-associated molecular patterns (DAMPs) and as receptors for pathogen-associated molecular patterns (PAMPs). **Sachiko Sato[1,2], Pampa Bhaumik[1,2], Christian St-Pierre[1,2], Valerie Meliot[1,2], Ann Rancourt[1,2], Michel Ouellet[1,2], Michel J. Tremblay[1,2].** [1]Research Centre for Infectious Diseases, CHUQ, Quebec, Canada; [2]Faculty of Medicine, Laval University, Quebec, Canada.

12:10 pm–12:30 pm 15 Functional roles of mammalian structural units, ligand cluster and polyvalency in the Abrus precatorius agglutinin and glycoprotein recognition process. **Albert M. Wu, June H. Wu, Jia-Hau Liu, Yuen-Yuen Chen, Biswajit Singha, Lu-Ping Chow, Jung-Yaw Lin.** Glyco-Immunochemistry Research Laboratory, Institute of Molecular and Cellular Biology, Chang-Gung University, Kwei-san, Taiwan; Department of Microbiology and Immunology, College of Medicine, Chang-Gung University, Kwei-san, Taiwan; The Institute of Biochemistry, College of Medicine, National Taiwan University, Taipei, Taiwan.

12:30 pm–12:50 pm 16 Diversity of carbohydrate binding profile among D-galactoside binding lectins isolated from lower animals. **Yuki Fujii[1] Ryo Matsumoto[1], Sakar M.A. Kawsar[1], Hidetaro Yasumitsu[1], Noriaki Kojima[2], Yasuhiro Ozeki[1].** [1]Yokohama City University, Yokohama, Japan; [2]YSFH, Yokohama, Japan.

12:50 pm–12:55 pm 17 Galectin-1 and HIV virion interaction: how does galectin-1 (but not galectin-3) selectively facilitates HIV infection in CD4+ cells? **Christian St-Pierre[1], Michel Ouellet[2], Tamao Endo[3], Garry F. Clarke[4], Hiroshi Manya[3], Michel J. Tremblay[2], Sachiko Sato[1].** [1]Glycobiology laboratory, Research Centre for Infectious Diseases, Faculty of Medicine, Laval University, Canada; [2]Laboratory of Human Immunoretrovirology, Research Centre for Infectious Diseases, Faculty of Medicine, Laval University, Canada; [3]Tokyo Metropolitan Institute of Gerontology, Tokyo, Japan; [4]University of Missouri-Columbia, MO, USA.

Concurrent Session 2: Structure/Function/Synthesis of Bioactive Oligosaccharides
Monday, 10:50 am–12:55 pm

Location: **San Geronimo Ball Room – A**
Co-Chairs: **Peter Orlean,** University of Illinois, USA
Henrik Clausen, University of Copenhagen, Denmark

10:50 am–11:10 am 18 Synthetic high-mannose-type glycans for analyses of glucosidase II and UDP-Glc:glycoprotein glucosyltransferase. **Yukishige Ito[1], Kiichiro Totani[1], Yoichi Takeda[1], Taisuke Watanabe[1], Atsushi Miyagawa[1], Ichiro Matsuo[1], Yoshito Ihara[2].** [1]RIKEN Advanced Science Institute; [2]Wakayama Medical University, Japan.

11:10 am–11:30 am 19 Side-Chain Conformations as Mediators of Saccharide Structure and Function: *N*-Acyl Groups in Aminosugars. **Anthony S. Serianni[1], Xiaosong Hu[1], Ian Carmichael[2].** [1]Department of Chemistry and Biochemistry, University of Notre Dame, Notre Dame, IN, USA; [2]Radiation Laboratory, University of Notre Dame, IN, USA.

11:30 am–11:50 am 20 Involvement of N-Acetylneuraminic Acid in the Regulation of GABA-Uptake Activity of GABA-Transporter 1. **Hua Fan[1], Jing Hu[1], Jian Fei[2], Pamela Stanley[3], Werner Reutter[1].** [1]Institut für Biochemie und Molekularbiologie, Charité-Universitätsmedizin Berlin, Germany; [2]Institute of Biochemistry and Cell Biology, SIBS, CAS, Shanghai, China; [3]Albert Einstein College of Medicine of Yeshiva University, Bronx, NY, USA.

11:50 am–12:10 pm 21 Shigella O-specific oligosaccharide-core-protein conjugates: new vaccine candidates. **Joanna Kubler-Kielb[1], Evgeny Vinogradov[2], Christopher Mocca[1], Chunyan Guo[1], John B. Robbins[1], Rachel Schneerson[1].** [1]National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD, USA; [2]Institute for Biological Sciences, National Research Council, Ottawa, ON, Canada.

12:10 pm–12:30 pm 22 Assay for the activity of C5-epimerase using engineered 2-O-sulfotransferase. **Kai Li, Heather N. Bethea, Jian Liu.** University of North Carolina at Chapel Hill, Chapel Hill, NC, USA.

12:30 pm–12:50 pm 23 Importance of differential N-glycosylation on a5ß1 integrin. **Jianguo Gu.** Tohoku Pharmaceutical University, Japan.

12:50 pm–12:55 pm 24 Total Synthesis Without Protecting Groups: Imino Sugars as Glycosidase Inhibitors. **Emma M. Dangerfield[1,2], Mattie S. M. Timmer[1,2], Bridget L. Stocker[1].** [1]Malaghan Institute of Medical Research; [2]Victoria University of Wellington, Wellington, New Zeland.

Concurrent Session 3: Biosynthesis, Regulation and Recognition of Pathogenic Glycans
Monday, 10:50 am–12:55 pm

Location: **San Geronimo Ball Room – C**
Co-Chairs: **D. Channe Gowda,** Penn Sate University, USA
Philip A. J. Gorin, Universidade Federal do Paraná Brazil

10:50 am–11:10 am 25 Hyaluronan in tissue remodeling. **Raija H. Tammi[1], Tiina Jokela[1], Anne Kultti[1], Kirsi Rilla[1], Hanna Siiskonen[1], Kari Törrönen[1], Sanna Pasonen-Seppänen[1], Päivi Auvinen[2], Maarit Anttila[2], Markku I. Tammi[1].** [1]University of Kuopio, Kuopio, Finland; [2]Kuopio University Hospital, Kuopio, Finland.

11:10 am–11:30 am 26 D-Arabinosyltransferases are druggable targets in tuberculosis: lessons learnt from Ethambutol. **Delphi Chatterjee, Jian Zhang.** Colorado State University, Fort Collins, CO, USA.

11:30 am–11:50 am 27 ß3GlcNAc-T5 induction in gastric epithelial cells by Helicobacter pylori leads to expression of sialyl-Lewis X, the ligand for SabA adhesin. **Celso A. Reis[1,2], Nuno T. Marcos[1], Ana Magalhães[1], Maria Oliveira[1], Filipe S. Silva[1,2], Ana S. Carvalho[1], Tim Gilmartin[3], Steven R. Head[3], Céu Figueiredo[1,2], Leonor David[1,2].** [1]Institute of Molecular Pathology and Immunology of the University of Porto, Portugal; [2]Medical Faculty of the University of Porto, Porto, Portugal; [3]The Scripps Research Institute, La Jolla, CA, USA.

11:50 am–12:10 pm 28 Glycan biosynthesis in the pathogenic fungus *Cryptococcus neoformans*. **Tamara L. Doering.** Washington University Medical School, St. Louis, MO, USA.

12:10 pm–12:30 pm 29 Characterisation of trans-sialidase genes from Trypanosoma congolense. **Sørge Kelm, Thaddeus T. Gbem, Hendrik Koliwer-Brandl, Frank Dietz.** Centre for Biomolecular Interactions Bremen, University Bremen, Bremen, Germany.

12:30 pm–12:50 pm 30 Initiation and Polymerization of Bacterial Polysialic Acids on Hydrophobic Acceptors. **Willie F. Vann[1], Justine Vionnet[1], Dwight C. Peterson[1], Gayathri Arakere[2].** [1]Center for Biologics Evaluation and Research/FDA, Bethesda, MD, USA; [2]Indian Institute of Science, Bangalore, India.

12:50 pm–12:55 pm 31 Analysis of Trypanosoma cruzi GPI10 — a multifunctional GPI mannosyltransferase. **John J. Scarcelli, Christopher H Taron.** New England Biolabs, Ipswich, MA, USA.

12:55 pm–2:05 pm **Lunch – *San Cristobal Ball Room***

Concurrent Session 4: Analyzing the Structure of Glycome
Monday, 2:10 pm–4:15 pm

Location: **San Geronimo Ball Room – B**
Co-Chairs: **Anthony S. Serianni,** University of Notre Dame, USA
Anne Dell, Imperial College London, UK

2:10 pm–2:30 pm 32 Lipopolysaccharide assembly in marine bacteria: definition of ensemble lipid heterogeneity by Vibrational Cooling Fourier Transform Mass Spectrometry. **Catherine E. Costello[1], Bogdan A. Budnik[1], Vera B. Ivleva[1], Svetlana V. Tomshich[2], Peter B. O'Connor[1], Yury N. Elkin[1,2].** [1]Mass Spectrometry Resource, Depatment of Biochemistry, Boston University School of Medicine, Boston, MA, USA; [2]Pacific Institute of Bioorganic Chemistry, Russian Academy of Sciences, Vladivostok, Russia.

2:30 pm–2:50 pm 33 Stable-isotope-assisted NMR approaches to structural glycomics. **Koichi Kato[1,2,3], Maho Yagi-Utsumi[1,2], Yukiko Kamiya[1], Takumi Yamaguchi[1], Yoshiki Yamaguchi[2,4].** [1]National Institute of Natural Science, Okazaki, Japan; [2]Nagoya City University, Nagoya, Japan; [3]Ochanomizu University, Tokyo, Japan; [4]RIKEN, Saitama, Japan.

2:50 pm–3:10 pm 34 New Approach for Glyco-and Lipidomics - Imaging Technology of Molecular Species of Brain Gangliosides by Combination of TLC-Blot and MALDI-TOF MS -. **Takao Taki[1], Tania Valdes Gonzarez[2], Hironobu Ishiyama[2], Naoko Goto-Inoue[3], Takahiro Hayasaka[3], Mitsutoshi Setou[3].** [1]Institute of Biomedical Innovation, Otsuka Pharmaceutical Co. Ltd., Tokushima, Japan; [2]Third Institute of Drug Discovery, Otsuka Pharmaceutical Co. Ltd., Tokushima, Japan; [3]Hamamatsu University School of Medicine, Shizuoka, Japan.

3:10 pm–3:30 pm 35 Latest advancements in HPLC-Chip/MS with applications to glycomics research. **Rudolf Grimm.** Agilent Technologies, Santa Clara, CA, USA.

3:30 pm–3:50 pm 36 A novel strategy for glycomic characterization of mucins using supported molecular matrix electrophoresis. **Yu-ki Matsuno[1], Weijie Dong[1], Takuro Saito[2], Mitsukazu Gotoh[2], Hisashi Narimatsu[1], Akihiko Kameyama[1].** [1]Research Center for Medical Glycoscience, National Institute of Advanced Industrial Science and Technology (AIST), Tsukuba, Japan; [2]Department of Surgery I, Fukushima Medical University, Fukushima, Japan.

3:50 pm–4:10 pm 37 Enrichment and Glycoproteomic Characterization of N- and O-glycopeptides from Cerebrospinal Fluid. **Jonas Nilsson, Adnan Halim, Camilla Hesse, Ulla Rüetschi, Göran Larson.** Department of Clinical Chemistry and Transfusion Medicine, Sahlgrenska University Hospital, Gothenburg, Sweden.

4:10 pm–4:15 pm 38 Current Challenges in Glycosylation Pattern Analysis of Snails. **Erika Staudacher[1], Herwig Stepan[1], Ahmed Aufy[1], Rudolf Geyer[2], Christina Bleckmann[2].** [1]University of Natural Resources and Applied Life Sciences, Vienna, Austria; [2]University of Giessen, Giessen, Germany.

Concurrent Session 5: Developmental Biology 1: Glycan in Vertebrate Development
Monday, 2:10 pm–4:15 pm

Location: **San Geronimo Ball Room – A**
Co-Chairs: **Kelley Moremen,** University of Georgia, USA
James, W. Dennis, Samuel Lunenfeld Research Institute, Canada

2:10 pm–2:30 pm 39 Glycosaminoglycans in Vertebrate Development: Form, Function and Future. **Rashmin C. Savani.** The University of Texas Southwestern Medical Center at Dallas, TX, USA.

2:30 pm–3:10 pm 215 The role of anti-gal antibodies in the development and progression of pre-malignant liver disease. **Anand S. Mehta, Lorena Loarca, Ronald Long, Mary Ann Comunale, Timothy M. Block.** Drexel University College of Medicine, Doylestown, PA, USA.

3:10 pm–3:30 pm 41 *O*-GlcNAc Protein Modification in Cancer Cells Increases in Response to Glucose Deprivation through Glycogen Degradation. **Jeong Gu Kang[1], Sang Yoon Park[1], Suena Ji[1], Insook Jang[1], Sujin Park[1], Hyun Sil Kim[2], Jong In Yook[2], Jürgen Roth[3], Jin Won Cho[1,3].** [1]Department of Biology; [2]Oral Pathology, Oral Cancer Research Institute, College of Dentistry; [3]WCU Program, Department of Biomecical Science, Graduate School, Yonsei University, Seodaemun-gu, Seoul, Korea.

3:30 pm–3:50 pm 42 Age-associated translocation of glycoprotein cathepsin D caused by oxidative stress and proteasome inhibition. **Tamao Endo, Yoko Sakurai, Yuri Miura.** Tokyo Metropolitan Institute of Gerontology, Tokyo, Japan.

3:50 pm–4:10 pm 43 A new function of polysialic acid on NCAM as the reservoir for the particular groups of neurotrophins, growth factors, and neurotransmitters that regulate the neural activity. **Ken Kitajima[1,2], Yukihiro Kanato[1], Ryo Isomura[1], Sayaka Ono[1], Chihiro Sato[1].** [1]Nagoya University, Nagoya, Japan; [2]Global COE on Systems Biology, Nagoya University, Japan.

4:10 pm–4:15 pm 44 Insights into neural cell metabolism from NMR. **Anika Gallinger[1], Hannelore Peters[1], Katja Karow[1], Luc Pellerin[2], Thomas Peters[1].** [1]University of Luebeck, Institute of Chemistry, Luebeck, Germany; [2]University of Lausanne, Lausanne, Switzerland.

Concurrent Session 6: Protein Folding, Targeting and Membrane Trafficking
Monday, 2:10 pm – 4:15 pm

Location: **San Geronimo Ball Room – C**
Co-Chairs: **Robert J. Wood,** University of Georgia, USA
Widmar Tanner, University of Regensburg, Germany

2:10 pm–2:30 pm 45 Protein N-glycosylation in yeast: New insights with surprises. **Ludwig Lehle, Katrin Hese, Michael Kämpf, Birgit Absmanner, Schmeiser Verena, Francoise Routier.** Universität Regensburg; Medizinische Hochschule Hannover.

2:30 pm–2:50 pm 46 Ligand undetectable membrane glycosphingolipids: cholesterol can make GSLs "invisible". **Clifford A. Lingwood.** Research Insitute, Hospital for Sick Children, Toronto, ON, Canada; Departments of Laboratory Medicine & Pathobiology, University of Toronto, Toronto, ON, Canada; Department of Biochemistry, University of Toronto, Toronto, ON, Canada.

2:50 pm–3:10 pm 47 Glycan-regulated proteolysis in the secretory pathway. **Richard N. Sifers.** Baylor College of Medicine, Houston, TX, USA.

3:10 pm–3:30 pm 48 Studies on the regulatory roles of the C-terminus of Cdc48 in the ERAD pathway. **Li Guangtao, Gang Zhao, William J. Lennarz.** State University of New York at Stony Brook, Stony Brook, NY, USA.

3:30 pm–3:50 pm 49 Apical Sorting by Galectin-3 Dependent Glycoprotein Clustering. **Ralf Jacob[1], Christoph Greb[1], Dominik Schneider[1], Annett Koch[1], Emma Salomonsson[2], Hakon Leffler[2], Andre LeBivic[3], Delphine Delacour[1].** [1]Department of Cell Biology and Cell Pathology, Philipps-Universität Marburg, Marburg, Germany; [2]Section MIG (Microbiology, Immunology, Glycobiology), Institute of Laboratory Medicine, Lund University, Lund, Sweden; [3]Laboratoire de Neurogenese et Morphogenese au cours du Developpement et chez l`Adulte (NMDA) / Institut de Biologie du Developpement de Marseille (IBDM), Faculte des Sciences de Luminy, Marseille, France.

3:50 pm–4:10 pm 50 The cytoplasmic PNGase-dependent ERAD pathway and free oligosaccharides (fOSs) in *Saccharomyces cerevisiae*. **Hiroto Hirayama[1], Akira Hosomi[1], Tadashi Suzuki[1,2].** [1]Glycometabolome Team, RIKEN Advanced Science Institute, Wako, Japan; [2]CREST, JST, Kawaguchi, Japan.

4:10 pm–4:15 pm 51 Structural basis of the molecular recognition by ERGIC-53 involved in the glycoprotein traffic in the cell. **Yukiko Kamiya[1,2], Miho Nishio[1,2], Tsunehiro Mizushima[2], Shoichi Wakatsuki[3], Kazuo Yamamoto[4], Susumu Uchiyama[5], Masanori Noda[5], Hans-Peter Hauri[6], Koichi Kato[1,2,7,8].** [1]National institutes of Natural Sciences, Okazaki, Japan; [2]Nagoya City University, Nagoya, Japan; [3]High Energy Accelerator Research Organization, Tsukuba, Japan; [4]The University of Tokyo, Kashiwa, Japan; [5]Osaka University, Osaka, Japan; [6]University of Basel, Basel, Switzerland; [7]Ochanomizu University, Tokyo, Japan; [8]Glyence Co., Ltd., Nagoya, Japan.

4:15 pm–4:30 pm Coffee/Tea Break - ***Foyer***

Plenary Lecture II (IGO Award Lecture)
Monday, 4:30 pm–5:30 pm

Location: **San Geronimo Ball Room – A, B & C**
Chair: **Paul Gleeson,** University of Melbourne, Australia

52 Markus Aebi. Institute of Microbiology, Department of Biology, ETH Zurich, Switzerland.
Concepts In N-Linked Protein Glycosylation

Poster Session I
Monday, 5:30 pm–7:30 pm

53 B1 Impaired expression of TLR in Trypanosoma cruzi infected Dendritic cells from galectin 3 defficient mice. **Pedro Bonay[1], Manuel Soto[1], Laura Corvo[1], Manuel Fresno[1], Miguel Angel Pineda[2].** [1]Centro de Biologia Molecular "Severo Ochoa"-Universidad Autonoma de Madrid, Madrid, Spain; [2]Division of Immunology, Infection and Inflammation. Glasgow University Research Centre, Glasgow, UK.

54 B2 Role of Galectins in the Innate Immune Response to Leishmaniasis. **Pampa Bhaumik, Christian St-Pierre, Sachiko Sato.** Laval University, Quebec City, Canada.

55 B3 Rat CD24 is one of major poly-N-acetyllactosamine-carrying glycoproteins in PC12 cells and rat bone marrow, but not in PC12D cells and rat brain. **Shigeyuki Fukui, Mizuho Kawahara.** Kyoto Sangyo University, Japan.

56 B4 Structural Characterization and Bioactivities of Hybrid Carrageenan-like Sulfated Galactan from Red Alga Furcellaria lumbricalis. **Guangli Yu[1], Bo Yang[1], Xia Zhao[1], Guanhua Du[2], Colin J. Barrow[3], H. Stephen Ewart[4], Junzeng Zhang[5].** [1]Glycoscience and Glycoengineering Laboratory, College of Medicine and Pharmacy, Ocean University of China, Qingdao, P. R. China; [2]National Center for Pharmaceutical Screening, Institute of Materia Medica, Chinese Academy of Medical Sciences & Peking Union Medical College, Beijing, P. R. China; [3]Ocean Nutrition Canada Ltd., Dartmouth, Nova Scotia, Canada; [4]Institute for Marine Biosciences, National Research Council Canada, Halifax, Nova Scotia, Canada; [5]Institute for Nutrisciences and Health, National Research Council Canada, Charlottetown, Prince Edward Island, Canada.

57 B5 Synthesis of Potential DNA Bisintercalators Based on Sugar Skelecton. **Jiang Tao, Li Wei, Ma Rui, Chen Shaopeng.** School of Pharmacy, Ocean University of China, China.

58 B6 Solid-phase synthesis of amino-functionalized glycoconjugates for attachment to solid surfaces and proteins. **Sara Spjut, Maciej Pudelko, Weixing Qian, Mikael Elofsson.** Department of Chemistry, University of Umeå, Umeå, Sweden.

59 B7 Probing the Structures and Interactions of Glucans with Proteins by Electrospray Mass Spectrometry and Carbohydrate Microarrays. **Wengang Chai, Yibing Zhang, Angelina S. Palma, Yan Liu, Ten Feizi.** Imperial College Faculty of Medicine, Northwick Park & St Marks Campus, London.

60 B8 Synthesis of glycosaminoglycan oligosaccharides equipped with fluorogenic groups for FRET. **Naoko Takeda, Miyuki Otaki, Remina Ikeda, Miwa Nanjo, Jun-ichi Tamura.** Tottori University, Tottori, Japan.

61 B9 Synthesis of primers for biosynthesis of proteoglycan. **Jun-ichi Tamura, Tomomi Nakamura, Ayaka Imazu.** Tottori University, Tottori, Japan.

62 B10 Design And Synthesis Of The Potential Transition State Analog Inhibitors Of Glycosyltransferases. **Jan Hirsch, Miroslav Koos, Igor Tvaroska.** Institute of Chemistry, Slovak Academy of Sciences.

63 B11 Synthesis Of Kdo-Human Serum Albumin Conjugate. **Tsuyoshi Ichiyanagi, Kazuhiko Kishi, Mayumi Fukunaga, Yoshinori Kawano.** Tottori University, Tottori, Japan.

64 B12 Aminoacyl saccharide as organocatalysts for asymetric aldol reaction. **Tomoya Machinami[1], Kazuya Sekihara[1], Ayumi Tsutsui[1], Kazuya Ito[1], Takayuki Kato[1], Kazuhito Sugawa[1], Noriko Sato[2], Takashi Fujimoto[1].** [1]Department of Chemistry, Meisei University, Japan; [2]School of Pharmaceutical Sciences, Kitasato University, Japan.

65 B13 Overexpression of MAN2C1 boosts ERAD and leads to accumulation of free oligomannosides. **Sandrine Duvet[1], Coralie Bernon[1], Yoann Carre[1], Marie-Christine Slomianny[1], Christian Slomianny[2], François Foulquier[1], Jean-Claude Michalski[1].** [1]UGSF UMR/CNRS 8576, USTL, Villeneuve d'Ascq, FRANCE; [2]Laboratoire de Physiologie Cellulaire - INSERM U800, USTL, Villeneuve d'Ascq, FRANCE.

66 B14 Proteomic analysis of GNA and RSA binding proteins in the pea aphid.
Els J.M. Van Damme[1], **Rameshwaram N. Rao**[1,2], **Mohamad Hamshou**[1,2], **Bart Ghesquière**[3,4], **Kris Gevaert**[3,4], **Guy Smagghe**[2]. [1]Laboratory of Biochemistry and Glycobiology, Department of Molecular Biotechnology, Ghent University, Gent, Belgium; [2]Laboratory of Agrozoology, Department of Crop Protection, Ghent University, Gent, Belgium; [3]Department of Medical Protein Research, VIB, Gent, Belgium; [4]Department of Biochemistry, Ghent University, Gent, Belgium.

67 B15 Transcript Analysis of Glycan-related Genes in Human Embryonic Stem Cells.
Alison V. Nairn, Mitche dela Rosa, Michael Kulik, Kazuhiro Aoki, Mindy Porterfield, Abigail Cline, Lance Wells, Stephen Dalton, Michael Tiemeyer, Kelley Moremen. University of Georgia, Athens, GA, USA.

68 B16 Ternary supra-molecular complex of oligosaccharyltransferase, Sec61 complex and ribosomes. **Yoichiro Harada, William J. Lennarz.** State University of New York at Stony Brook, Stony Brook, NY, USA.

69 B17 Monitoring Anti-Angiogenic Action of Tunicamycin by Raman Spectroscopy.
Maria O. Longas[1], **Ashok Kotapati**[1], **Kilari PVRK. Prasad**[2], **Aditi Banerjee**[3], **Dipak K. Banerjee**[3].
[1]Purdue University Calumet, Department of Chemistry and Physics, Hammond, IN, USA;
[2]Purdue University Calumet, Department of Computer and Information Technology, Hammond, IN, USA; [3]Department of Biochemistry, School of Medicine, University of Puerto Rico, San Juan, PR, USA.

17 B18 Galectin-1 and HIV virion interaction: how does galectin-1 (but not galectin-3) selectively facilitates HIV infection in CD4+ cells? **Christian St-Pierre**[1], **Michel Ouellet**[2], **Tamao Endo**[3], **Garry F. Clarke**[4], **Hiroshi Manya**[3], **Michel J. Tremblay**[2], **Sachiko Sato**[1]. [1]Glycobiology laboratory, Research Centre for Infectious Diseases, Faculty of Medicine, Laval University, Canada; [2]Laboratory of Human Immunoretrovirology, Research Centre for Infectious Diseases, Faculty of Medicine, Laval University, Canada; [3]Tokyo Metropolitan Institute of Gerontology, Tokyo, Japan; [4]University of Missouri-Columbia, MO, USA.

24 B19 Total Synthesis Without Protecting Groups: Imino Sugars as Glycosidase Inhibitors.
Emma M. Dangerfield[1,2], **Mattie S. M. Timmer**[1,2], **Bridget L. Stocker**[1]. [1]Malaghan Institute of Medical Research; [2]Victoria University of Wellington, Wellington, New Zeland.

31 B20 Analysis of Trypanosoma cruzi GPI10 — a multifunctional GPI mannosyltransferase.
John J. Scarcelli, Christopher H Taron. New England Biolabs, Ipswich, MA, USA.

38 B21 Current Challenges in Glycosylation Pattern Analysis of Snails. **Erika Staudacher**[1], **Herwig Stepan**[1], **Ahmed Aufy**[1], **Rudolf Geyer**[2], **Christina Bleckmann**[2]. [1]University of Natural Resources and Applied Life Sciences, Vienna, Austria; [2]University of Giessen, Giessen, Germany.

44 B22 Insights into neural cell metabolism from NMR. **Anika Gallinger**[1], **Hannelore Peters**[1], **Katja Karow**[1], **Luc Pellerin**[2], **Thomas Peters**[1]. [1]University of Luebeck, Institute of Chemistry, Luebeck, Germany; [2]University of Lausanne, Lausanne, Switzerland.

51 B23 Structural basis of the molecular recognition by ERGIC-53 involved in the glycoprotein traffic in the cell. **Yukiko Kamiya[1,2], Miho Nishio[1,2], Tsunehiro Mizushima[2], Shoichi Wakatsuki[3], Kazuo Yamamoto[4], Susumu Uchiyama[5], Masanori Noda[5], Hans-Peter Hauri[6], Koichi Kato[1,2,7,8].** [1]National institutes of Natural Sciences, Okazaki, Japan; [2]Nagoya City University, Nagoya, Japan; [3]High Energy Accelerator Research Organization, Tsukuba, Japan; [4]The University of Tokyo, Kashiwa, Japan; [5]Osaka University, Osaka, Japan; [6]University of Basel, Basel, Switzerland; [7]Ochanomizu University, Tokyo, Japan; [8]Glyence Co., Ltd., Nagoya, Japan

228 (LB-1) B24 Effect of N-glycans on structural stability of arylphorin. **Soohyun Kim[1], Kyoung-Seok Ryu[1]**. [1]Korea Basic Science Institute, Daejeon, South Korea

234 (LB-7) B25 Dendritic cells previously exposed to mannan-binding lectin (MBL) enhance cytokine production in allogeneic mononuclear cell cultures.**David C. Kilpatrick[1], Shirley L. MacDonald[2], Ian Downing[3], Anne PM. Atkinson[4], Marc L. Turner[5]**. [1]Scottish National Blood Transfusion Service, Edinburgh, U.K., [2]Scottish National Blood Transfusion Service, Edinburgh, U.K., [3]Scottish National Blood Transfusion Service, Edinburgh, U.K., [4]Scottish National Blood Transfusion Service, Edinburgh, U.K., [5]Scottish National Blood Transfusion Service, Edinburgh, U.K.

236 (LB-9) B26 Notch Xylosyltransferases. **Hans Bakker, Maya K. Sethi, Falk FR. Buettner, Vadim B. Krylov, Hideyuki Takeuchi, Nikolay E. Nifantiev, Robert S. Haltiwanger, Rita Gerardy-Schahn.** [1]Hannover Medical School, Germany, [2]Hannover Medical School, Germany, [3]Hannover Medical School, Germany, [4]Zelinsky Institute of Organic Chemistry, Moscow, Russian Federation, [5] Stony Brook University, NY, USA, [6]Zelinsky Institute of Organic Chemistry, Moscow, Russian Federation, [7]Stony Brook University, NY, USA, [8]Hannover Medical School, Germany.

243 (LB-15) B27 Impact of N-glycosylation on glucagon receptor function **Anita Johswich[1], Christine Longuet[1], Judy Pawling[1], Michael Ryczko[1], Daniel Drucker[1], James W. Dennis[1]**. [1]Samuel Lunenfeld Research Institute, Mount Sinai Hospital, Toronto, ON, Canada.

246 (LB-19) B28 The GT2 enzyme NodC as a model for studying processive chitin synthase**.** **Helge C. Dorfmueller and Daan M.F. van Aalten.** Division of Molecular Microbiology, College of Life Sciences, University of Dundee, Scotland, UK

Tuesday, December 1, 2009

7:00 am–6:30 pm	**Registration**
6:30 am–8:25 am	**Breakfast –** ***San Cristobal Ball Room***

Sunrise Session II: Systems Biology of Glycans: Order Emerging from Glycoform Chaos
Tuesday, 8:30 am–9:45 am

Location:	**San Gerenimo Ball Room – A, B & C**
Chair:	**Johannes F.G. Vliegenthart,** Utrecht University, The Netherlands

8:30 am–8:50 am 70 Evolutionary trends in protein N-glycosylation. **James W. Dennis[1,2], Ryan J Williams[1].** [1]Samuel Lunenfeld Research Institute; [2]University of Toronto, Toronto, ON, Canada.

8:55 am–9:15 am 247 ER-to-cytosol protein dislocation: from one into another chaos. **Jurgen Roth**. Department of Biomedical Science, Yonsei University Graduate School, Yonsei University, Seoul, Korea

9:20 am–9:40 am 72 Role of "Glycan cycle" in disease ; lessons from functional glycomics. **Naoyuki Taniguchi[1,2].** [1]Department of Disease Glycomics, Institute of Science & Industrial Research, Osaka University, Ibaraki, Japan; [2]Systems Glycobiology Research Group, Advanced Science Institute, RIKEN, Saitama, Japan.

Plenary Lecture III
Tuesday, 9:45 am–10:30 am

Location: **San Geronimo Ball Room – A, B & C**
Chair: **Vincent C. Hascall,** Cleveland Clinic, USA

73 Jukka Finne
University of Helsinki, Helsinki, Finland; University of Turku, Turku, Finland
Absence of polysialic acid - an unfavorable prognostic marker of advanced stage neuroblastoma.

10:30 am–10:50 am Coffee/Tea Break - ***Foyer***

Concurrent Session 7: Glycoengineering and Industrial Applications
Tuesday, 10:50 am–12:55 pm

Location: **San Geronimo Ball Room – A**
Co-Chairs: **Ram Sasisekharan,** MIT, USA
Leo März, Universität für Bodenkultur, Austria

10:50 am–11:10 am 77 Glyco-engineering in plants: Production of monoclonal antibodies containing homogeneously β1,4-galactosylated human-type N-glycans. **Josef Glössl[1], Richard Strasser[1], Alexandra Castilho[1], Renate Kunert[2], Friedrich Altmann3, Lukas Mach[1], Herta Steinkellner[1].** [1]Department of Applied Genetics and Cell Biology, BOKU University, Vienna, Austria; [2]Department of Biotechnology, BOKU University, Vienna, Austria; [3]Department of Chemistry, BOKU University, Vienna, Austria

11:10 am–11:30 am 75 Chemical Protein Glycosylation: A new Approach to Protein Stabilization. **Kai H. Griebenow.** Department of Chemistry, University of Puerto Rico, Rio Piedras, PR, USA.

11:30 am–11:50 am 76 Glycan analysis of a plant-cell derived Glucocerebrosidase as a tool for monitoring changes in growth condition and manufacturing. **Yoram Tekoah, Yehava Forster, Yoseph Shaaltiel, David Aviezer.** Protalix Biotherapeutics, Carmiel, Israel.

11:50 am–12:10 pm 74 Remodeling of N-glycosylation pathway of the methylotrophic yeast Hansenula polymorpha: Evaluation of the ALG3 deletion strain blocked in the lipid-linked oligosaccharide assembly as a host for the production of therapeutic glycoproteins. **Hyun Ah Kang.** Department of Life Science, Chung-Ang University, Dongjak-gu, Seoul, Korea

12:10 pm–12:30 pm 78 Engineering a New Class of Carbohydrate-binding Proteins: Computationally Guided Mutagenesis of O-GlcNAcase. **Nina E. Weisser[1], Elisa Fadda[1], Loretta Yang[1], Robert J. Woods[1,2].** [1] School of Chemistry, National University of Ireland, Galway, Galway, Ireland; [2]Complex Carbohydrate Research Center, Department of Biochemistry and Molecular Biology, University of Georgia, Georgia, USA.

12:30 pm–12:50 pm 79 Overexpression of ST6GalNAcV, a ganglioside-specific α2,6 sialyltransferase, inhibits glioma invasivity. **Roger A. Kroes[1], Mary E. Schmidt[1], Huan He[2], Mark R. Emmett[2], Alan G. Marshall[2], Carol L. Nilsson[2], Joseph R. Moskal[1].** [1]The Falk Center for Molecular Therapeutics, Department of Biomedical Engineering, Northwestern University, Evanston, IL, USA; [2]National High Magnetic Field Laboratory, Florida State University, Tallahassee, FL, USA.

12:50 pm–12:55 pm 80 A combined method for producing homogeneous glycoproteins with eukaryotic N-glycosylation. **Flavio Schwarz[1], Wei Huang[2], Cishan Li[2], Benjamin L. Schulz[1], Christian Lizak[1], Shin Numao[1], Markus Aebi[1], Lai-Xi Wang[2].** [1]ETH Zurich, Zurich, Switzerland; [2]University of Maryland School of Medicine, Baltimore, MD, USA.

Concurrent Session 8: Glycobiology of Human Diseases
Tuesday, 10:50 am–12:55 pm

Location: **San Geronimo Ball Room – B**
Co-Chairs: **Willie F. Vann**, Center for Biologics Evaluation and Research/ FDA, USA
Chitra Mandal, Indian Institute of Chemical Biology, India

10:50 am–11:10 am 81 An Update on Congenital Disorders of Glycosylation. **Thierry Hennet, Micha Haeuptle, Charlotte Maag, Andreas Hülsmeier.** University of Zürich, Switzerland.

11:10 am–11:30 am 82 Modeling Glycosylation Disorders in Zebrafish: New Insights into Pathophysiology. **Richard Steet, Ningguo Gao, Mark Lehrman, Heather Flanagan-Steet.** University of Georgia, Athens, GA, USA; UT-Southwestern Medical Center, Dallas, TX, USA; UT-Southwestern Medical Center, Dallas, TX, USA; University of Georgia, Athens, GA, USA.

11:30 am–11:50 am 83 The COG Complex Functions In Trafficking Of Glycosyltransferases Through The Golgi. **Richard D. Smith[1], Tetyana Kudlyk[1], Irina Pokrovskaya[1], Rose Willett[1], Willy Morelle[2], Vladimir Lupashin[1].** [1]University of Arkansas for Medical Sciences, Little Rock, AR, USA; [2]University of Lille, Lille, France.

11:50 am–12:10 pm 84 Hyperglycemia induces intracellular hyaluronan synthesis and autophagy in dividing rat mesangial cells. **Aimin Wang, Juan Ren, Vincent Hascall.** Department of Biomedical Engineering, Lerner Research Institute, Cleveland Clinic, Cleveland, OH, USA.

12:10 pm–12:30 pm 85 Role of SHAP-hyaluronan complex in ovalbumin-induced airway hyperresponsiveness in mice. **Lisheng Zhuo[1], Long Zhu[2], Koji Kimata[1,2], Etsuro Yamaguchi[3], Kenji Baba[3].** [1]Research Complex for the Medicine Frontiers, Aichi Medical University, Japan; [2]Institute for Molecular Science of Medicine, Aichi Medical University, Japan; [3]Department of Internal Medicine, Aichi Medical University, Japan.

12:30 pm–12:50 pm 86 Diagnostic accuracy of serum glycoprotein levels and fucosylation for the differential diagnosis of liver diseases measured by ELISA. **Bishnu P. Chatterjee, Gautam Mondal, Urmimala Chatterjee.** West Bengal University of Technology, Kolkata, India.

12:50 pm–12:55 pm 87 Identification of new soluble oligosaccharide structures in CDG-I deficient cells. **Francois Foulqier[1], Wendy Vleugels[2], Sandrine Duvet[1], Anne-Marie Mir[1], Jean-claude Michalski[1], Gert Matthijs[2].** [1]UMR 8576 Structural and Functional Glycobiology Unit, Villeneuve D'Ascq 59655, France; [2]Laboratory for Molecular Diagnosis, Center for Human Genetics, University of Leuven, Belgium.

Concurrent Session 9: Plant Glycobiology
Tuesday, 10:50 am–12:55 pm

Location: **San Geronimo Ball Room – C**
Co-Chairs: **Alan D. Elbein,** University of Arkansas, USA
Daan van Aalten, University of Dundee, UK

10:50 am–11:10 am 88 Carbohydrate-binding proteins in the nucleocytoplasmic compartment of plant cells. **Els J.M. Van Damme, Nausicaä Lannoo, Dieter Schouppe, Gianni Vandenborre, Elke Fouquaert, Jonas Van Hove.** Laboratory of Biochemistry and Glycobiology, Department of Molecular Biotechnology, Ghent University, Coupure Links, Gent, Belgium.

11:10 am–11:30 am 89 Expression of recombinant human Lewis fucosyltransferase III in plant. **Guy G. Costa[1], Caroline C. Le Morvan[1], Renaud R. Leonard[2], Sabine S. Lhernould[1].** [1]Faculté des Sciences et Techniques, Laboratoire de Chimie des Substances Naturelles, Equipe de Glycobiologie Forestière, Limoges Cedex, France; [2]Institut für Chemie, Universität für Bodenkultur, Vienna, Austria.

11:30 am–11:50 am 93 Cellulase assays with the region-specifically substituted p-nitrophenyl-β-D-glucopyranoside and cellobioside on the glycon portion. **Takeshi Nishimura, Mitsuro Ishihara.** Forestry and Forest Products Research Institute, Tsukuba, Ibaraki, Japan

11:50 am–12:10 pm 91 Molecular cloning and characterization of glucosyltransferases from Gerbera hybrida. **Anja Lampio[1,2], Teemu Teeri[2]** [1]University of Technology, Espoo, Finland; [2]University of Helsinki, Helsinki, Finland.

12:10 pm–12:30 pm 92 Computational Analysis of the Strength of Water-Protein Interactions in Concanavalin A. **Elisa Fadda[1], Robert J. Woods[1,2].** [1]School of Chemistry, National University of Ireland, Galway, Galway, Ireland; [2]Complex Carbohydrate Research Center, University of Georgia, Athens, GA, USA.

12:30 pm–12:50 pm 90 Role of β-N-acetylhexosaminidases in the formation of paucimannosidic N-glycans in plants. **Josef Glössl[1], Eva Liebminger[1], Jennifer Schoberer[1], Barbara Svoboda[1], Friedrich Altmann[2], Herta Steinkellner[1], Lukas Mach[1], Richard Strasser[1].** [1]Department of Applied Genetics and Cell Biology, BOKU University, Vienna, Austria; [2]Department of Chemistry, BOKU University, Vienna, Austria.

12:50 pm–12:55 pm 94 Complexation of 1,6-Anhydro-maltooligosaccharides and their Glycoconjugates. **Takayuki Kato[1], Yui Honma[1], Kazuhito Sugawa[1], Takashi Fujimoto[1], Noriko Sato[2], Mitsuru Tashiro[1], Tomoya Machinami[1].** [1]Department of Chemistry, Meisei University, Japan; [2]School of Pharmaceutical Sciences, Kitasato University, Japan.

12:55 pm–2:05 pm **Lunch –** ***San Cristobal Ball Room***

Concurrent Session 10: Developmental Biology II: Glycans in Lower- or Non-Vertebrate Development and Cell Survival
Tuesday, 2:10 pm – 4:15 pm

Location: **San Geronimo Ball Room - B**
Co-Chairs: **Rashmin C. Savani,** UT Southwestern Medical Center, USA
Monica M. Palcic, Carlsberg Laboratory, Denmark

2:10 pm–2:30 pm 96 Functional Roles of Sperm Hyaluronidases, HYAL5 and SPAM1, in Mouse Fertilization. **Tadashi Baba.** University of Tsukuba, Ibaraki, Japan.

2:30 pm–2:50 pm 95 Investigating the Roles of Mucin-Type O-Glycosylation During Eukaryotic Development. **Kelly G. Ten Hagen, Liping Zhang, Duy Tran, E Tian, Zhang Xuefeng.** Developmental Glycobiology Unit, NIDCR, National Institutes of Health, Bethesda, MD, USA.

2:50 pm–3:10 pm 97 MicroRNAs regulate Heparan sulfate proteoglycan's function. **Kan Ding[1], Xiaokun Shen[1], Songshan Jiang[2].** [1]Chinese Academy of Sciences, Shanghai, China; [2]Sun Yat-Sen University, Guangzhou, China.

3:10 pm–3:30 pm 98 Site directed processing: role of amino acid sequences and glycosylation of acceptor glycopeptides in the assembly of extended mucin type O-glycan core 2. **Inka Brockhausen[1], Thomas R. Dowler[1], Hans Paulsen[2].** [1]Department of Medicine, Biochemistry, Queen's University, Kingston ON, Canada; [2]Department of Chemistry, University Hamburg, Hamburg, Germany.

3:30 pm–3:50 pm 99 Enhancement / suppression of the activity of bovine testicular and human cancer cell hyaluronidases by formation of hyaluronan-protein complexes. **Brigitte Deschrevel[1], Trias Astériou[1], Hélène Lenormand[1], Frédéric Tranchepain[1], Jean-Claude Vincent[1], Bertrand Delpech[1,2].** [1]Laboratory "Polymères, Biopolymères, Surfaces", University of Rouen - CNRS, Mont Saint Aignan, France; [2]Laboratory MERCI, EA 2122 – Centre Hospitalier Universitaire, University of Rouen, Rouen, France.

3:50 pm–4:10 pm 100 Characterization of glucosylceramides in Scedosporium apiospermum and their involvement in fungal differentiation. **Eliana Barreto-Bergter[1], Rodrigo Rollin-Pinheiro[1], Guilherme L. Sassaki[2], Lauro M. Souza[2], Livia Cristina L. Lopes[1], Philip AJ. Gorin[2].** [1]Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brasil; [2]Universidade Federal do Paraná, Curitiba, Paraná, Brasil.

4:10 pm–4:15 pm 101 Novel UDP-Gal derivative interferes with active site closure and inhibits transfer in human blood group glycosyltransferases. **Rene Jorgensen[1], Thomas Pesnot[2], Gerd Wagner[2], Monica Palcic[1].** [1]Carlsberg Laboratory, Valby, Denmark; [2]University of East Anglia, Norwich, UK.

Concurrent Session 11: Glycans of Embryonic and Adult Stem Cells
Tuesday, 2:10 pm–4:15 pm

Location: **San Geronimo Ball Room – A**
Co-Chairs: **John Magnani,** GlycoMimetics, USA
Tamao Endo, Tokyo Metropolitan Institute of Gerontology, Japan

2:10 pm–2:30 pm 102 The sugar paved road towards improved stem cell therapy. **Suvi Natunen, Leena Valmu.** Finnish Red Cross Blood Service, Helsinki, Finland.

2:30 pm–2:50 pm 103 Role of polysialic acid and NCAM in postnatal neurogenesis. **Gerardy-Schahn Rita, Roeckle Iris, Muehlenhoff Martina, Weinhold Birgit, Hildebrandt Herbert.** Hannover Medical School, Hannover, Germany.

2:50 pm–3:10 pm 104 Linking glycan expression to pathway dynamics during stem cell differentiation. **Michael Tiemeyer, Kazuhiro Aoki, Mindy Porterfield, Alison Nairn, Jae-Min Lim, Meng Fang, Michael Kulik, Lance Wells, Stephen Dalton, Kelley Moremen.** The Complex Carbohydrate Research Center, The University of Georgia, Athens, GA,USA.

3:10 pm–3:30 pm 105 Quantitative Glycomics During Cellular Differentiation. **Maho Amano[1], Misa Yamaguchi[1], Yasuhiro Takegawa[1], Tadashi Yamashita[1], Jun-Ichi Furukawa[1], Michiyo Terashima[2], Norimasa Iwasaki[2], Akio Minami[2], Yoshiaki Miura[1], Shin-Ichiro Nishimura[1].** [1]Graduate School of Advance Life Sciences, Hokkaido University, Sapporo, Japan; [2]Graduate School of Medicine, Hokkaido University, Sapporo, Japan.

3:30 pm–3:50 pm 106 Expression and possible functions of gangliosides in neural stem cells. **Makoto Yanagisawa, Robert K. Yu.** Institute of Molecular Medicine and Genetics and Institute of Neuroscience, Medical College of Georgia, Augusta, GA, USA.

3:50 pm–4:10 pm 107 The 3'-phosphoadenosine 5'-phosphosulfate transporters, PAPST1 and PAPST2, are important for the maintenance and the differentiation of mouse embryonic stem cells. **Shoko Nishihara[1], Norihiko Sasaki[1], Takuya Hirano[1], Tomomi Ichimiya[1], Masahiro Wakao[2], Hidenao Toyoda[3], Yasuo Suda[2].** [1]Depertment of Bioinfomatics, Soka University, Hachioji, Tokyo, Japan; [2]Graduate School of Science and Engineering, Kagoshima University, Kohrimoto, Kagoshima, Japan; [3]College of Pharmaceutical Sciences, Ritsumeikan University, Kusatsu, Shiga, Japan.

4:10 pm–4:15 pm 108 Cryptic glycan epitopes in necrotic mesenchymal stem cells. **Virve Pitkänen[1], Tia Hirvonen[1], Sari Tiitinen[1], Suvi Natunen[1], Petri Lehenkari[1,2], Saara Laitinen[1], Leena Valmu[1].** [1]Finnish Red Cross Blood Service, Research and Development, Helsinki, Finland; [2]University of Oulu, Finland.

Concurrent Session 12: Glycan Biosynthesis, Structure and Regulation I
Tuesday, 2:10 pm–4:15 pm

Location: **San Geronimo Ball Room – C**
Co-Chairs: **Subroto Chatterjee,** Johns Hopkins University, USA
David Rose, University of Waterloo, Canada

2:10 pm–2:30 pm 109 Glycosphingolipid storage diseases and tuberculosis: The unexpected connection. **Fran M. Platt.** Department of Pharmacology, University of Oxford, UK.

2:30 pm–2:50 pm 110 Myelin-associated Glycoprotein (Siglec 4) Protects Axons from Acute Toxicity via a Ganglioside-dependent Mechanism. **Niraj R. Mehta, Thien Nguyen, John W. Griffin, Ronald L. Schnaar.** The Johns Hopkins School of Medicine, Baltimore, MD, USA.

2:50 pm–3:10 pm 111 UDP-sugar precursors infuence hyaluronan synthesis by substrate availability and HAS transcription in keratinocytes. **Markku I. Tammi[1], Tiina Jokela[1], Katri Makkonen[1], Elina Koli[1], Carsten Carlberg[1,2], Raija H. Tammi[1], Kari Törrönen[1].** [1]University of Kuopio, Kuopio, Finland; [2]University of Luxembourg, Luxembourg.

3:10 pm–3:30 pm 112 Targeting an antimicrobial effector function in insect immunity as a pest control strategy. **Rahul Raman[1,2,3], Mark S. Bulmer[4], Ido Bachelet[1,2,3], Rebeca B. Rosengaus[4], Ram Sasisekharan[1,2,3].** [1]Harvard-MIT Division of Health Sciences and Technology; [2]Koch Institute of Integrative Cancer Research; [3]Department of Biological Engineering, Massachusetts Institute of Technology, Cambridge, MA, USA; [4]Department of Biology, Northeastern University, Boston, MA, USA.

3:30 pm–3:50 pm 113 The Motility Of Ovarian Carcinoma Cells Is Regulated Through A Glycosphingolipid/Caveolin-1 Signaling Complex. **Alessandro Prinetti[1], Massimo Aureli[1], Giuditta Illuzzi[1], Simona Prioni[1], Valentina Nocco[1], Federica Scandroglio[1], Giovanni Tredici[2], Virginia Rodriguez-Menendez[2], Vanna Chigorno[1], Sandro Sonnino[1].** [1]Department of Medical Chemistry, Biochemistry and Biotechnology, University of Milan, Milan, Italy; Department of Neuroscience and Biomedical Technologies, [2]University of Milan-Bicocca, Milan, Italy.

3:50 pm–4:10 pm 114 Segregation of Mucin Core 2 Enzymes, C2GnT-1(L) and C2GnT-2(M), in the Golgi Apparatus. **Pi-Wan Cheng, Mohamed Ali.** University of Nebraska Medical Center, Omaha, NE, USA.

4:10 pm–4:15 pm 115 Cholesterol depletion suppresses hyaluronan synthesis by downregulating hyaluronan synthase 2 associated with inhibition of Akt and activation of STAT5. **Anne Kultti[1], Riikka Kärnä[1], Kirsi J. Rilla[1], Katri Makkonen[1,2], Pertti Nurminen[1], Elina Koli[1], Markku I. Tammi[1], Raija H. Tammi[1].** [1]Institute of Biomedicine, Anatomy, University of Kuopio, Kuopio, Finland; [2]Department of Biosciences, Biochemistry, University of Kuopio, Kuopio, Finland.

4:15 pm–4:30 pm **Coffee/Tea Break –** ***Foyer***

Plenary Lecture IV – San Geronimo Ball Room
Tuesday, 4:35 pm–5:20 pm

Location: **San Geronimo Ball Room – A, B & C**
Chair: **Johannis P. Kamerling,** Utrecht University, The Netherlands

116 Geert-Jan Boons
Complex Carbohydrate Research Center, University of Georgia, Athens, GA, USA.
Modulation of Biological Functions with Synthetic Carbohydrate-Based Macromolecules.

Poster Session II
Tuesday, 5:30 pm–7:30 pm

117 B1 Functional characterization of *Yarrowia lipolytica* homologues of *Saccharomyces cerevisiae MNN4* and *MNN6* genes in mannosylphosphorylation of *N*- and *O*-linked olig. **Jeong-Nam Park[1], Yunkyoung Song[3], Seon Ah Cheon[1], Ohsuk Kwon[2], Doo-Byoung Oh[2], Jeong-Yoon Kim[3], Hyun Ah Kang[1].** [1]Department of Life Science, Chung-Ang University, Seoul, Korea; [2]Integrative Omics Research Center, Korea Research Institute of Bioscience and Biotechnology, Daejeon, Korea; [3]School of Bioscience and Biotechnology, Chungnam National University, Daejeon, Korea.

118 B2 Glycosylation Prevents Moisture-Induced Instabilities of alpha-Chymotrypsin in the Solid State. **Giselle M Flores-Fernandez, Miraida Pagan, Mariangely Almenas, Kai H. Griebenow.** Department of Chemistry, University of Puerto Rico - Rio Piedras Campus, San Juan, PR, USA.

119 B3 On the role of protein structural dynamics in the catalytic activity and thermostability of serine protease subtilisin Carlsberg. **Ricardo J. Solá, Kai Griebenow.** Department of Chemistry, University of Puerto Rico - Rio Piedras Campus, San Juan, PR, USA.

120 B4 O-mannosyl glycosylation is involved in assembly of the basement membrane. **Huaiyu Hu, Yuan Yang, Christine Gagen, Peng Zhang.** SUNY Upstate Medical University, Syracuse, NY, USA.

121 B5 Comprehensive Glycan Analysis of Alpha-1-Antitrypsin in Hepatitis C Induced Liver Cirrhosis and Cancer. **Mary Ann Comunale, Lucy Betesh, Mengjun Wang, Mehta Anand.** Drexel University College of Medicine, Dolyestown, PA, USA.

122 B6 Genetic Alteration of Mannosylphospho Dolichol Synthase Differentially Regulates Angiogenesis. **Zhenbo Zhang[1], Aditi Banerjee[1], Isamarie Frontany[1], Krishna Baksi[2], Dipak K. Banerjee[1].** [1]Department of Biochemistry, School of Medicine, University of Puerto Rico, San Juan, PR, USA; [2]Department of Anatomy and Cell Biology, School of Medicine, Universidad Central del Caribe, Bayamon, PR, USA.

123 B7 Study on novel α-*N*-acetylglucosaminidases: hydrolytic activity and substrate specificity. **Akiko Tsuchida[1], Masaya Fujita[1], Kohtaro Goto[1], Yuriko Hirose[1], Hisashi Ashida[2], Katsuji Haneda[3], Jun Nakayama[4], Mamoru Mizuno[1].** [1]The Noguchi Institute, Tokyo, Japan; [2]Kyoto University, Kyoto, Japan; [3]Kanagawa Institute Technology, Atsugi, Japan; [4]Shinshu University School of Medicine, Matsumoto, Japan.

124 B8 Simultaneous determination of nucleotide sugars with ion-pair reversed-phase HPLC and LC-ESI-MS. **Kazuki Nakajima[1], Shinobu Kitazume[2], Eiji Miyoshi[3], Naoyuki Taniguchi[1,2].** [1]Department of Disease Glycomics, Institute of Science & Industrial Research, Osaka University, Ibaraki; [2]Systems Glycobiology Research Group, Advanced Science Institute, RIKEN, Saitama, Japan; [3]Department of Molecular Biochemistry & Clinical Investigation, Osaka University Graduate School of Medicine, Suita, Japan.

125 B9 Monoclonal Antibodies To Human Polypeptide GalNAc-T14. **A. Sjoberg, K. Kato, E. P. Bennett, H. H. Wandall, H. Clausen, U. Mandel.** Center for Glycomics, School of Dentistry and Department of Cellular and Molecular Medicine, University of Copenhagen, Copenhagen, Denmark.

126 B10 N-Glycans modulate the activation of a common cytokine signal transducer, gp130, in mouse embryonic neural stem cells. **Makoto Yanagisawa, Robert K. Yu.** IMMAG, Medical College of Georgia, Augusta, GA, USA.

127 B11 Sugars, Stable Isotopes, and Spectrometry: New Methods for the Analysis of Carbohydrate Metabolism. **Neil P. J. Price, Karl Vermillion.** USDA National Center Agricultural Utilization Research, NCAUR-ARS-USDA, Peoria, IL, USA.

128 B12 Immobilization of *Helicobacter pylori* α1,3-fucosyltransferase on magnetic beads *via* C-terminal membrane anchoring region. **Kentaro Naruchi, Shin-Ichiro Nishimura.** Hokkaido University, Sapporo, Japan.

129 B13 Automated N-Glycan Composition Analysis with LC-MS/MS. **Ilja Ritamo[1], Hannu Peltoniemi[2], Jarkko Räbinä[1], Leena Valmu[1].** [1]Finnish Red Cross Blood Service, Helsinki, Finland; [2]Applied Numerics Oy, Helsinki, Finland.

130 B14 Sequence analysis of endo-α-N-acetylgalactosaminidases. **Daniil G. Naumoff.** Laboratory of Bioinformatics, State Institute for Genetics and Selection of Industrial Microorganisms, Moscow, Russia.

80 B15 A combined method for producing homogeneous glycoproteins with eukaryotic N-glycosylation. **Flavio Schwarz[1], Wei Huang[2], Cishan Li[2], Benjamin L. Schulz[1], Christian Lizak[1], Shin Numao[1], Markus Aebi[1], Lai-Xi Wang[2].** [1]ETH Zurich, Zurich, Switzerland; [2]University of Maryland School of Medicine, Baltimore, MD, USA.

87 B16 Identification of new soluble oligosaccharide structures in CDG-I deficient cells. **Francois Foulqier[1], Wendy Vleugels[2], Sandrine Duvet[1], Anne-Marie Mir[1], Jean-claude Michalski[1], Gert Matthijs[2].** [1]UMR 8576 Structural and Functional Glycobiology Unit, Villeneuve D'Ascq 59655, France; [2]Laboratory for Molecular Diagnosis, Center for Human Genetics, University of Leuven, Belgium.

94 B17 Complexation of 1,6-Anhydro-maltooligosaccharides and their Glycoconjugates. **Takayuki Kato[1], Yui Honma[1], Kazuhito Sugawa[1], Takashi Fujimoto[1], Noriko Sato[2], Mitsuru Tashiro[1], Tomoya Machinami[1].** [1]Department of Chemistry, Meisei University, Japan; [2]School of Pharmaceutical Sciences, Kitasato University, Japan.

101 B18 Novel UDP-Gal derivative interferes with active site closure and inhibits transfer in human blood group glycosyltransferases. **Rene Jorgensen[1], Thomas Pesnot[2], Gerd Wagner[2], Monica Palcic[1].** [1]Carlsberg Laboratory, Valby, Denmark; [2]University of East Anglia, Norwich, UK.

108 B19 Cryptic glycan epitopes in necrotic mesenchymal stem cells. **Virve Pitkänen[1], Tia Hirvonen[1], Sari Tiitinen[1], Suvi Natunen[1], Petri Lehenkari[1,2], Saara Laitinen[1], Leena Valmu[1].** [1]Finnish Red Cross Blood Service, Research and Development, Helsinki, Finland; [2]University of Oulu, Finland.

115 B20 Cholesterol depletion suppresses hyaluronan synthesis by downregulating hyaluronan synthase 2 associated with inhibition of Akt and activation of STAT5. **Anne Kultti[1], Riikka Kärnä[1], Kirsi J. Rilla[1], Katri Makkonen[1,2], Pertti Nurminen[1], Elina Koli[1], Markku I. Tammi[1], Raija H. Tammi[1].** [1]Institute of Biomedicine, Anatomy, University of Kuopio, Kuopio, Finland; [2]Department of Biosciences, Biochemistry, University of Kuopio, Kuopio, Finland.

227 B21 Studies on eukaryotic sialic acid O-acetylation. **Roland Schauer[1], Gnanapragassam V. Srinivasan[1,2]**. [1]University of Kiel, Kiel, Germany, [2]University of Nebraska, Omaha, NE, USA.

229 (LB-2) B22 Glycoengineering of human Growth Hormone**. Thomas Veje Lundgaard[1,2], Peter Thygesen[3], Henrik Clausen[2], Claus Kristensen[1], Gert Bolt[1].** [1]Mammalian Cell Technology, Biopharmaceuticals Research Unit, Novo Nordisk A/S, Maaloev, Denmark, [2]Copenhagen Center for Glycomics, Department of Cellular and Molecular Medicine, University of Copenhagen, Copenhagen, Denmark, [3]Exploratory ADME, Biopharmaceuticals Research Unit, Novo Nordisk A/S, Maaloev, Denmark

231 (LB-4) B23 Plasma protein glycation association in diabetes rats. **Chitta R. Sahu**, Department of Zoology, University of Kalyani, Kalyani, India.

239 (LB-12) B24 NMR Spectroscopy to Elucidate the Interaction between the Transmembrane Receptor CCR5 and Glycopeptides from the GP120 of HIV **Bernd Meyer**. University of Hamburg, Hamburg, Germany.

241 (LB-14) B25 The Leu125Val and Ser563Asn Gene Polymorphisms Combination of Platelet Endothelial Cell Adhesion Molecule-1(PECAM-1) increases Endothelial Function**. Heming Wei[1], Lu Feng[1], Bin Huang[1], Subroto Chatterjee[1].** [1]Department of Pediatrics Johns Hopkins University, School of Medicine, Baltimore, MD, USA

Wednesday, December 2, 2009

8:30 am–11:30 am **Registration**

6:30 am–8:25 am **Breakfast –** ***San Cristobal Ball Room***

Sunrise Session III: Cell Survival and Cell Death: Differential Splicing of a Common Glycan Pathway
Wednesday, 8:30 am–9:45 am

Location: **San Geronimo Ball Room – A, B & C**
Chair: **Catherine E. Costello,** Boston University School of Medicine, USA

8:30 am–8:50 am 131 Glycosaminoglycan-protein interactions in innate immunity and inflammation. **Anthony J. Day[1], Simon J. Clark[1], Issac Zambrano[2], Svetlana Hakobyan[3], Paul Morgan[3], Paul N. Bishop[1].** [1]University of Manchester, UK; [2]The Manchester Eye Banck, UK; [3]Cardiff University, UK.

8:55 am–9:15 am 132 Cancer Biomarker Discovery – Exploring The O-Glycoproteome For Biomarkers. **H. H. Wandall[1], O. Blixt[1], M. A. Tarp[1], J. W. Pedersen[1], E. P. Bennett[1], U. Mandel[1], G. Ragupathi[2], P. Livingston[2], M. A. Hollingsworth[3], J. Taylor-Papadimitriou[4], J. Burchell[4], H. Clausen[1].** [1]Center for Glycomics, University of Copenhagen, Copenhagen, Denmark; [2]Department of Medicine, Memorial Sloan-Kettering Cancer, New York, NY, USA; [3]Eppley Institute for Research in Cancer, University of Nebraska Medical Center, Omaha, NE, USA; [4]King's College London School of Medicine, Guy's Hospital London, London,UK.

9:20 am–9:40 am 133 O-Glycan Functions in Development and Cancer. **Cummings D. Cummings, Tongzong Ju, Yingchun Wang, Jean Phillipe Gourdine, Rongjuan Mi, Sean Stowell, David Smith, Qian Sun, Rajindra Aryal, Amy Wang.** Department of Biochemistry, Emory University School of Medicine, Atlanta, GA, USA.

Plenary Lecture V
Wednesday, 9:45 am–10:30 am

Location: **San Geronimo Ball Room – A, B & C**
Chair: **Joseph R. Moskal,** Northwestern University, USA

134 Subhash C. Basu
Department of Chemistry and Biochemistry, University of Notre Dame, Notre Dame, IN, USA.
Biosynthesis of Glycolipids and Its Regulations In Apoptotic Carcinoma Cells

10:30 am–10:50 am **Coffee/Tea Break -** ***Foyer***

Concurrent Session 13: Glycan Biosynthesis, Structure and Regulation II
Wednesday, 10:50 am–12:55 pm

Location: **San Geronimo Ball Room - A**
Co-Chairs: **John Hanover,** National Institutes of Health, USA
Alessandro Prinetti, University of Milan, Italy

10:50 am–11:10 am 135 Blood Group Glycosyltransferases: Structure and Function of Natural and Unnatural Mutants. **Monica M. Palcic.** Carlsberg Laboratory, Copenhagen, Denmark.

11:10 am–11:30 am 136 Genetic and biochemical analysis of the biosynthesis and assembly of the glycan N-linked to flagellins of the archaeon Methanococcus maripaludis. **Ken F. Jarrell.** Queen's University, Kingston, ON, Canada.

11:30 am–11:50 am 137 Cyberinfrastructure For Glycome Research. **Arun K. Datta.** National University, La Jolla, CA, USA

11:50 am–12:10 pm 138 The Conversion of Trehalose to Glycogen: A New Pathway for Synthesis of Glycogen. **Alan D. Elbein, Irena Pastuszak, Y.T. Pan.** Department of Biochemistry and Molecular Biology, University of Arkansas for Medical Sciences, Little Rock, AR, USA.

12:10 pm–12:30 pm 139 Noncanonical Asn-linked glycosylation occurs on recombinant Heat shock protein 60 secreted from Chinese hamster ovary cells. **Lei Zhou[1,2], Xiaojing Yun[1,2], Jianxin Gu[1,2].** [1]Key Laboratory of Glycoconjugate Research, Ministry of Health, PR. China; [2]Department of Biochemistry and molecular biology, Shanghai Medical College, Fudan University, PR. China.

12:30 pm–12:50 pm 140 Functional and structural analysis reveals dual function on C-terminal alpha-helix of Alg13 protein. **Xiao-Dong Gao, Satoru Moriyama, Nobuaki Miura, Shin-Ichiro Nishimura.** Hokkaido University, Sapporo, Japan.

12:50 pm–12:55 pm 141 LOX-1, as a receptor, cross-presents Hsp60-fused antigen on MHC class I molecules. **Jianhui Xie[1,2], Jianxin Gu[1,2].** [1]Key Laboratory of Glycoconjugate Research, Ministry of Health, PR. China; [2]Department of Biochemistry and molecular biology, Shanghai Medical College, Fudan University, PR. China.

Concurrent Session 14: Glycans of Pathogenic Viruses, Parasites and Microorganisms
Wednesday, 10:50 am–12:55 pm

Location: **San Geronimo Ball Room – C**
Co-Chairs: **Tom Oeltman,** Vanderbilt University School of Medicine, USA
Vladimir M. Lakhtin, G.N. Gabrichevsky Intitute of Epidemiology and Microiology, Russia

10:50 am–11:10 am 142 Highly pathogenic avian H5N1 viruses that acquire human receptor specificity. **Yasuo Suzuki.** Chubu University, Kasugai-shi, Aichi, Japan.

11:10 am–11:30 am 143 Structural basis of carbohydrate receptor recognition by viruses. **Thilo Stehle[1,2].** [1]University of Tuebingen, Tübingen, Germany; [2]Vanderbilt University School of Medicine, Nashville, TN, USA.

11:30 am–11:50 am 144 Novel fucose binding lectin domain from the human pathogen *Bukholderia cenocepacia*. **Ondrej Sulak[1,2], Gianluca Cioci[3], Monia Delia[1], Anne Imberty[2], Michaela Wimmerova[1,4].** [1]National Centre for Biomolecular Research, Faculty of Science, Masaryk University, Brno, Czech Republic; [2]CERMAV-CNRS, BP 53, F-38041 Grenoble cedex 09, France; [3]ESRF, Experiments Division, BP 220, 38043 Grenoble, France; [4]Department of Biochemistry, Faculty of Science, Masaryk University, Brno, Czech Republic.

11:50 am–12:10 pm 200 O-GlcNAc Modification on E-cadherin repressor Snail confers Epithelial-Mesenchymal Transition. **Sang Yoon Park[1], Hyun Sil Kim[2], Suena Ji[1], Jeong Gu Kang[1], Jong In Yook[2], Jin Won Cho[1,3].** [1]Department of Biology; [2]Oral Pathology, Oral Cancer Research Institute, College of Dentistry; [3]WCU program, Department of Biomecical Science, Graduate School, Yonsei University, Seoul, Korea

12:10 pm–12:30 pm 146 An inhibitor of galactosyltransferases alters the cell surface properties of lung cancer cells. **Inka Brockhausen[1], Xiaojing Yang[1] Kathleen Newmarch[1], Walter A. Szarek[2].** [1]Department of Medicine and Biochemistry, Queen's University, Kingston ON, Canada; [2]Department of Chemistry, Queen's University, Kingston ON, Canada.

12:30 pm–12:50 pm 147 Human noroviruses recognize α1,2-fucosylated glycosphingolipids on thin-layer chromatograms and in supported lipid bilayers. **Gustaf E. Rydell[1], Jonas Nilsson[1], Andreas B. Dahlin[2], Jacques Le Pendu[3], Fredrik Höök[2], Göran Larson[1].** [1]Sahlgrenska University Hospital, Gothenburg, Sweden; [2]Chalmers University of Technology, Gothenburg, Sweden; [3]Université de Nantes, Nantes, France.

12:50 pm–12:55 pm 148 A Novel Shigella Galactosyltransferase With Unusual Properties Involved In B14 O-Antigen Synthesis. **Changchang Xu[1], Walter A. Szarek[1], Inka Brockhausen[2], Bin Liu[2], Bo Hu[2], Yanfang Han[2], Lu Feng[2], Lei Wang[2].** [1]Queen's University, Kingston, ON, Canada; [2]Nankai University, Tianjin, China.

Concurrent Session 15: Glyconanotechnology and Bioinformatics
Wednesday, 10:50 am–12:55 pm

Location:	**San Geronimo Ball Room – B**
Co-Chairs:	**Stephan Ladisch,** Children's National Medical Center, USA
	Fran, M. Platt, University of Oxford, UK

10:50 am–11:10 am 149 Integrated Approach to Glycomics. **Ram Sasisekharan[1,2,3], Rahul Raman[1,2,3].** [1]Harvard-MIT Division of Health Sciences and Technology, Cambridge, MA, USA [2]Koch Institute of Integrative Cancer Research, Cambridge, MA, USA; [3]Department of Biological Engineering, Massachusetts Institute of Technology, Cambridge, MA, USA.

11:10 am–11:30 am 150 Mimicking the carbohydrate-carbohydrate cell recognition in marine sponge cells. **Johannis P. Kamerling[1], Adriana Carvalho de Souza[1], Henricus J. Vermeer[1], Simon R. Haseley[1], Johannes FG. Vliegenthart[1], J Ignacio Santos[2], F Javier Cañada[2], Sonsoles Martín-Santamaria[3], Jesús Jiménez-Barbero[2].** [1]Bijvoet Center, Bio-Organic Chemistry, Utrecht University, Utrecht, Netherlands; [2]Centro de Investigaciones Biológicas, CIB-CSIC, Madrid, Spain; [3]Departomento de Química, Facultad de Farmacia, Universidad San Pablo CEU, Madrid, Spain.

11:30 am–11:50 am 151 Computational Prediction of Influenza Receptor Specificity. **Robert J. Woods.** University of Georgia, Complex Carbohydrate Research Center, Athens, GA, USA.

11:50 am–12:10 pm 152 Conjugated Tunicamycin Nanoparticles and Nanotubes for Breast Cancer Therapy. **Karen T. Johnson[1], Millie L. Gonzalez[2], Josie Fuentes[2], Nilmarie Ayala[2], Aditi Banerjee[3], Jesus Santiago[3], Carmen J. Hernandez[2], Ipsita A. Banerjee[1], Dipak K. Banerjee[3].** [1]Department of Chemistry, Fordham University, Bronx, NY, USA; [2]Department of Biology, University of Puerto Rico, Humacao, PR, USA; [3]Department of Biochemistry, University of Puerto Rico-Rio Piedras Campus, San Juan, PR, USA.

12:10 pm–12:30 pm 153 Glycosignature analysis of complex glycans. **Sergei A. Svarovsky.** The Biodesign Institute, Tempe, AZ, USA.

12:30 pm–12:50 pm 154 Bacterial Carbohydrate Structure Database version 3. **Philip V. Toukach.** N.D. Zelinsky Institute of Organic Chemistry, Russian Academy of Sciences, Moscow, Russia.

12:50 pm–12:55 pm 155 Japan Consortium for Glycobiology and Glycotechnology DataBase. **Toshihide S. Shikanai[1], Yohichi S. Shimma[1], Yoshinori S. Suzuki[1], Noriaki F. Fujita[1], Hiroyuki K. Kaji[1], Takashi S. Sato[1], Akira T. Togayachi[1], Akihiko K. Kameyama[1], Hiroaki T. Tateno[1], Jun Hirabayashi[1], Shujiro Okuda[2], Toshisuke Kawasaki[2,10], Noriko Takahashi[3], Koichi Kato[3], Koichi Furukawa[4,10], Etsuko Yasugi[5], Masahiro Nishijima[5], Kiyoko Kinoshita[6], Shoko Nishihara[6,10], Issaku Yamada[7], Mamoru Mizuno[7], Takashi Shirai[7], Masaki Kato[8], Yoshiki Yamaguchi[8], Eriko Hagiya[9], Keiichi Yoshida[8,10], Naoyuki Taniguchi[8,10], Hisashi Narimatsu[1,10].** [1]Research Center for Medical Glycoscience, National Institute of Advanced Industrial Science; [2]Ritsumeikan University; [4]Nagoya City University; [4]Nagoya University; [5]Executive Committee of Lipid Database; [6]Soka University; [7]Noguchi Institute; [8]RIKEN; [9]Seikagaku Corporation; [10]JCGG, Japan.

Afternoon Off (Lunch on your own)

Thursday, December 3, 2009

8:30 am–5:30 pm **Registration**

6:30 am–8:25 am **Breakfast –** ***San Cristobal Ball Room***

Sunrise Session IV: Staying Young and Getting Old: Are Glycans a Curse or a Blessing?
Thursday, 8:30 am–9:45 am

Location: **San Geronimo Ball Room - A, B & C**
Chair: **Roland Schauer**, University of Kiel, Germany

8:30 am–8:50 am 156 Nothing in Sialobiology Makes Sense, Except in the Light of Evolution. **Ajit Varki.** University of California-San Diego, La Jolla, CA, USA.

8:55 am–9:15 am 157 GPI-glycan remodeling by PGAP5 regulates transport of GPI-anchored proteins from the ER to the Golgi. **Taroh Kinoshita[1], Yusuke Maeda[1], Moonjin Ra[2], Yoshiki Yamaguchi[3], Ryo Taguchi[2], Morihisa Fujita[1].** [1]Osaka University, Osaka, Japan; [2]University of Tokyo, Tokyo, Japan; [3]RIKEN, Saitama, Japan.

9:20 am–9:40 am 158 N-linked glycosylation in plants; Curse or Blessing for whom? **Dirk Bosch[1,2], Maurice Henquet[1], Sander van der Krol[3].** [1]Plant Research International, Wageningen UR, [2]The Netherlands; Department of Chemistry, Utrecht University, The Netherland; [3]Laboratory of Plant Physiology, Wageningen University, The Netherlands.

Plenary Lecture VI
Thursday, 9:45 am–10:30 am

Location: **San Geronimo Ball Room – A, B & C**
Chair: **Angela M. Gronenborn,** University of Pittsburgh School of Medicine, USA

159 James H. Prestegard
University of Georgia, Athens, GA, USA
Isotopic labeling and NMR Characterization of Glycans on Glycoproteins

10:30 am–10:50 am Coffee/Tea Break - ***Foyer***

Concurrent Session 16: Protein-Carbohydrate Interaction
Thursday, 10:50 am–12:55 pm

Location: **San Geronimo Ball Room – A**
Co-Chairs: **Rudolf Grimm**, Agilent Technologies, USA
Els J.M. Van Damme, Ghent University, Belgium

10:50 am–11:10 am 160 The CVNH family of lectins — structure, folding and sugar binding. **Angela M. Gronenborn.** University of Pittsburgh School of Medicine, Pittsburgh, PA, USA.

11:10 am–11:30 am 161 Structural basis for differences in carbohydrate specificities among members of Jacalin Related Lectin (JRL) family. **Avadhesha Surolia[1,2], Garima Gupta[2], Mamannamana Vijayan[2].** [1]National Institute of Immunology, New Delhi, India; [2]Molecular Biophysics Unit, Indian Institute of Science, Bangalore, India.

11:30 am–11:50 am 162 Auto-antibodies to glycans: repertoire, specificity and proposed role in immunity. **Nicolai V. Bovin.** Shemyakin Institute of Bioorganic Chemistry, RAS, Moscow, Russia.

11:50 am–12:10 pm 163 Chondroitin sulfate proteoglycan-mediated adherence of *Plasmodium falciparum*-infected red blood cells in human placenta. **D. Channe Gowda, Suchi Goel, Atul Goyal.** Penn State University College of Medicine, Hershey, PA, USA.

12:10 pm–12:30 pm 164 The role of carbohydrates in viral infections – A Nuclear Magnetic Resonance (NMR) study. **Thomas Haselhorst.** Griffith University, Gold Coast, Queensland, Australia.

12:30 pm–12:50 pm 165 Binding of Porcine Submaxillary Mucin Analogs to Lectins. **Curtis F. Brewer[1], Tarun K. Dam[1], Thomas A. Gerken[2].** [1]Albert Einstein College of Medicine, Bronx, NY, USA; [2]Case Western Reserve University School of Medicine, Cleveland, OH, USA.

12:50 pm–12:55 pm 166 Syndecan-4 regulates the cell-surface trafficking and activity of pro-fibrotic factor Transglutaminase-2. **Alessandra Scarpellini[1], Hugues Lortat-Jacob[2], Timothy Johnson[3], Elisabetta AM. Verderio[1].** [1]Nottingham Trent University, Nottingham, UK; [2]Institut de Biologie Structurale CNRS-CEA-UJF, Grenoble, France; [3]Academic Nephrology Unit, Medical School, University of Sheffield, Sheffield, UK.

Concurrent Session 17: Carbohydrate-Carbohydrate Interaction
Thursday, 10:50 am – 12:55 pm

Location: **San Geronimo Ball Room – C**
Co-Chairs: **Robert K. Yu,** Medical College of Georgia, USA
Koichi Kato, National Institute of Natural Sciences, Japan

10:50 am–11:10 am 167 Probing carbohydrate-carbohydrate interactions with multivalent glycoconjugates. **Amit Basu.** Brown University, RI, USA.

11:10 am–11:30 am 168 Carbohydrate-mediated cell adhesion involved in pathogenic homing behaviors of T- and B-lymphocytes. **Reiji Kannagi[1], Keiichiro Sakuma[1], Keiko Miyazaki[1], Naoko Kimura[1], Katsuyuki Ohmori[2].** [1]Department of Molecular Pathology, Aichi Cancer Center, Nagoya, Japan; [2]Division of Cell Analysis, Kyoto University Hospital, Kyoto, Japan.

11:30 am–11:50 am 169 Carbohydrate-Carbohydrate Interactions Between Myelin Glycosphingolipids. **Joan M. Boggs[1,2], Wen Gao[1], Jingsha Zhao[3], Amit Basu[3].** [1]Hospital for Sick Children, Toronto, ON, Canada; [2]University of Toronto, Toronto, ON, Canada; [3]Brown University, Providence, RI, USA.

11:50 am–12:10 pm 170 Carbohydrate Tumor Antigen Vaccines using Novel Strategies. **Kate Rittenhouse-Olson[1], Padmini Sahoo[1], Susan Morey[1], Amy Houghton[1], Andreas Sungren[2], Joseph Barchi[2].** [1]University at Buffalo, Buffalo, NY, USA; [2]National Cancer Institute, Frederick, MD, USA.

12:10 pm–12:30 pm 171 Stability of human plasma N-glycome. **Gordan Lauc[1,3], Olga Gornik[1], Jasenka Wagner[2], Ana Knezevic[1], Maja Pucic[3], Irma Redzic[1].** [1]University of Zagreb, Faculty of Pharmacy and Biochemistry, Zagreb, Croatia; [2]University of Osijek, School of Medicine, Osijek, Croatia; [3]Genos Ltd., Glycobiology Division, Zagreb, Croatia.

12:30 pm–12:50 pm 172 A new route to labeled oligosaccharides via glycosyl azides in aqueous media. **Atsushi Kobayashi, Hikaru Nagai, Tomonari Tanaka, Masato Noguchi, Masaki Ishihara, Shin-ichiro Shoda.** Graduate School of Engineering, Tohoku University, Sendai, Japan.

12:50 pm–12:55 pm 173 Immunological disorder analysis of polylactosmaine synthase-deficient mice. **Akira Togayachi[1], Yuzuru Ikehara[1], Hiroyasu Ishida[1,2], Yuko Kozono[1], Hideki Matsuzaki[1], Takashi Sato[1], Takashi Kudo[3], Satoru Takahashi[3], Jun Hirabayashi[1], Hisashi Narimatsu[1].** [1]Research Center for Medical Glycoscience (RCMG), AIST, Ibaraki, Japan; [2]Institute of Clinical Medicine, University of Tsukuba, Ibaraki, Japan; [3]Institute of Basic Medical Sciences, University of Tsukuba, Ibaraki, Japan.

Concurrent Session 18: Glycobiology of Cancer and Glycotherapeutics
Thursday, 10:50 am–12:55 pm

Location: **San Geronimo Ball Room – B**
Co-Chairs: **Ajit Varki,** University of California, USA
Zhengmei Zhu, Dalian Medical University, China

10:50 am–11:10 am 174 Mapping the substrate binding subsites of Golgi &[alpha]-Mannosidase II and implications for inhibitor development. **David R. Rose[1,2,3], Niket Shah[2,3], Douglas A. Kuntz[3]**. [1]University of Waterloo, Waterloo, ON, Canada, [2]University of Toronto, Ontario, Canada, [3]Ontario Cancer Institute, Toronto, ON, Canada.

11:10 am–11:30 am 175 Heparin upregulates mir-10b targeting Hoxd10 and inhibit angiogenesis. **Kan Ding[1], Xiaokun Shen[1], Songshan Jiang[2].** [1]Chinese Academy of Sciences, Shanghai, China; [2]Sun Yat-Sen University, Guangzhou,, China.

11:30 am–11:50 am 176 Tunicamycin Inhibits Angiogenesis and Breast Tumor Growth by Modulating the Cell Survival Signal. **Aditi Banerjee[1], Krishna Baksi[2], Dipak K. Banerjee[1].** [1]Department of Biochemistry, School of Medicine, Univerity of Puerto Rico, San Juan, PR, USA; [2]Department of Anatomy & Cell Biology, Universidad Central del Caribe, Bayamon, PR, USA.

11:50 am - 12:10 pm 177 Distinct roles of neural cell specific carbohydrates, polysialic acid and HNK-1 glycan, in cell-cell interaction and tumor formation. **Minoru Fukuda[1], Kiyohiko Angata[1], Misa Suzuki[1], Isabelle Franceschini[2].** [1]Burnham Institute for Medical Research, La Jolla, CA, USA; [2]National Institute for Agriculture, Tours, France.

12:10 pm - 12:30 pm 178 Glycosyltransferase gene expression manipulation as a therapeutic strategy for the treatment of malignant brain tumors. **Jospeh R. Moskal[1], Roger A. Kroes[1], Glyn Dawson[2].** [1]Northwestern University, Evanston, IL, USA; [2]University of Chicago, Chicago, IL, USA.

12:30 pm–12:50 pm 179 Inhibition of U937-Cell Adhesion to Human Endothelial Cells by Glycosylated Lysoyzme Mutants. **Ralph Melcher[1], Stefanie Demmig[1], Dorothea Moeller[1], Ines Kraus[2], Theodor Kudlich[1], Hardi Luehrs[1], Michael Scheurlen[1], Andrej Hasilik[2].** [1]Department of Medicine II, University Hospital of Wuerzburg, Wuerzburg, Germany; [2]Institute for Physiological Chemistry, Phillips University of Marburg, Marburg, Germany.

12:50 pm–12:55 pm 180 Role of N-acetylglucosaminyltransferase III and V in the Post-Translational Modifications of E-cadherin. **Salomé S. Pinho[1,2], Celso A. Reis[1,3], Joana Paredes[1], Ana Maria Magalhães[1], António Carlos Ferreira[1], Joana Figueiredo[1], Wen Xiaogang[1,3], Fátima Carneiro[1,3], Fátima Gartner[1,2], Raquel Seruca[1,3].** [1]Institute of Molecular Pathology and Immunology of the University of Porto (IPATIMUP), Portugal; [2]Institute of Biomedical Sciences of Abel Salazar (ICBAS), Portugal; [3]Medical Faculty, University of Porto, Portugal.

12:55 pm–2:05 pm Lunch – ***San cristobal Ball Room***

Concurrent Session 19: Cell Signaling
Thursday, 2:10 pm–4:15 pm

Location: **San Geronimo Ball Room – A**
Co-Chairs: **Roger A. Kroes**, Northwestern University, USA
Jürgen Roth, Yonsei University, Korea

2:10 pm–2:30 pm 181 Carbohydrate microarrays toward deciphering the glycome. **Ten Feizi.** The Glycosciences Laboratory, Imperial College London, Harrow, Middlesex, UK.

2:30 pm–2:50 pm 182 Cell signaling functions of C-mannosylated peptides from the thrombospondin type 1 repeat. **Yoshito Ihara[1], Shino Manabe[2], Yoko Inai[1], Midori Ikezaki[1], Yukishige Ito[2].** [1]Wakayama Medical University, Wakayama, Japan; [2]RIKEN Advanced Science Institute, Saitama, Japan.

2:50 pm–3:10 pm 183 Modulation of cell functions by glycosphingolipid metabolic remodeling in the plasma membrane. **Sandro Sonnino, Vanna Chigorno, Alessandro Prinetti, Massimo Aureli.** Università degli Studi di Milano, Milan, Italy.

3:10 pm–3:30 pm 184 Sialic acid biosynthesis is involved in proliferation and gene expression. **Rüdiger Horstkorte[1], Christian Klukas[2], Andreas Klein[3], Andreas Simm[1], Falk Schreiber[2], Wenke Weidemann[1].** [1]Martin-Luther-Universität, Halle-Wittenberg, Germany; [2]Leibniz Institute of Plant Genetics and Crop Plant Research (IPK) Gatersleben, Germany; [3]Charité, Campus Benjamin Franklin, Germany.

3:30 pm–3:50 pm 185 Pathophysiological Effects of Anti-lipid A Antibody on Sodium Channel Activities: Implication on Guillain-Barré Syndrome. **Robert K. Yu, Seigo Usuki.** Medical College of Georgia, Augusta, GA, USA.

3:50 pm–4:10 pm 186 O-Glycopeptide Specific Auto-Antibodies For Early Detection of Colorectal Cancer. **J. W. Pedersen[1], O. Blixt[1], E. P. Bennett[1], M. A. Tarp[1], I. Dar[1], A. E. Pedersen[2], S. S. Poulsen[3], U. Mandel[1], H. Clausen[1], H. H. Wandall[1].** [1]Center for Glycomics, [2]Department of International Health, Immunology and Microbiology, [3]Department of Biomedicine, and Faculty of Health Sciences, University of Copenhagen, Copenhagen, Denmark.

4:10 pm–4:15 pm 187 Glycosylation of voltage-gated potassium channels affects neuronal expression and localization. **Desiree A. Thayer, Lily Y. Jan.** University of California, San Francisco, CA, USA.

Concurrent Session 20: Structural and Chemical Glycobiology
Thursday, 2:10 pm–4:15 pm

Location: **San Geronimo Ball Room – C**
Co-Chairs: **Michael Pierce,** University of Georgia Cancer Center, USA
Naoyuki Taniguchi, Osaka University, Japan

2:10 pm–2:30 pm 188 Molecular mechanisms of O-GlcNAc signalling. **Daan van Aalten.** University of Dundee, Dundee, UK.

2:30 pm–2:50 pm 189 Dynamic Crosstalk Between GlcNAcylation & Phosphorylation: Roles in Signaling & Human Disease. **Gerald W. Hart, Chad Slawson, Zihao Wang, Pui Butkinaree, Kaoru Sakabe, Kyoungsook Park, Shino Shimoji, Quira Zeidan, John Bullen, Ron Copeland.** Johns Hopkins University School of Medicine, Baltimore, MD, USA.

2:50 pm–3:10 pm 190 Hexosamine Signaling: Friend or Foe in Feast or Famine. **John A. Hanover, Salil Ghosh, Chithra Keembiyehetty, Peng Wang, Marcy Comley, Gayani Weersinghe, Michelle Mondoux, Michael Krause, Dona Love.** NIDDK, National Institutes of Health, Bethesda, MD, USA

3:10 pm–3:30 pm 191 Structure-Function Studies and Design of Novel Glycosyltransferases for Site Specific Bioconjugation of mAbs and scFv via Glycan residues: Development of a Targeted Drug Delivery System and Contrast Agents for MRI. **Pradman K. Qasba[1], Boopathy Ramakrishnan[1,2], Elizabeth Boeggeman[1,2], Marta Pasek[1], Maria Manzoni[1].** [1]Structural Glycobiology Section, Center for Cancer Research, NCI, National Institutes of Health, Frederick, MD, USA; [2]Basic Research Program, SAIC-Frederick, Inc., Center for Cancer Research, Nanobiology Program, NCI, National Institutes of Health, Frederick, MD, USA.

3:30 pm–3:50 pm 192 Oligosaccharide components of the Glycoprotein of the Opportunistic Pathogen *Scedosporium prolificans*. **Philip A. J. Gorin[1], Guilherme L. Sassaki[1], Lauro M. Souza[1], Ricardo Wagner[1], Miguel D. Noseda[1], Fernanda S. Tosin[1], Mariana I.D. da Silva[2] , Vera C.B. Bittencourt[2], Eliana Barreto-Bergter[2].** [1]Universidade Federal do Paraná, Curitiba-PR, Brazil; [2]Universidade Federal do Rio de Janeiro, Rio de Janeiro-RJ, Brazil.

3:50 pm–4:10 pm 193 *In silico* and *in vitro* binding studies predict a novel sialic acid binding site on the sialic acid binding Ig-like lectin-7 (Siglec-7). **Nao Yamakawa[1,2,3], Hitomi Kosaki[1,2], Paul R. Crocker[4], Gérard Vergoten[5], Chihiro Sato[1,2,3], Ken Kitajima[1,2,3].** [1]Graduate School of Bioagricultural Sciences, Nagoya University, Nagoya, Japan; [2]Bioscience and Biotechnology Center, Nagoya University, Nagoya, Japan; [3]Global COE Systems Biology, Nagoya University, Nagoya, Japan; [4]College of Life Sciences, University of Dundee, UK; [5]University of Science & Technology, Unité Glycobiologie Structurale et Fonctionnelle, Lille, France.

4:10 pm–4:15 pm 194 Novel aspects of the catalytic mechanism of human blood group B galactosyltransferase from NMR and microcalorimetry. **Nora Sindhuwinata[1], Hannelore Peters[1], Thies Koehli[1], Eva Munoz[1,2], Thomas Weimar[1], Monica M. Palcic[3], Thomas Peters[1].** [1]University of Luebeck, Institute of Chemistry, Luebeck, Germany; [2]University of Santiago de Compostela, Compostela, Spain; [3]Carlsberg Laboratory, Valby, Denmark.

Concurrent Session 21: Translational Glycobiology
Thursday, 2:10 pm–4:15 pm

Location: **San Geronimo Ball Room – B**
Co-Chairs: **Kai H. Griebenow,** University of Puerto Rico, USA
Takao Taki, Otsuka Pharmaceutical Company, Japan

2:10 pm–2:30 pm 195 Ganglioside metabolism, the tumor microenvironment, and tumor progression. **Stephan Ladisch.** Children's National Medical Center, Washington, DC, USA.

2:30 pm–2:50 pm 196 Therapeutic approaches for Tay-Sachs and Sandhoff disease models with recombinant human lysosomal β-hexosaminidase. **Kohji Itoh[1,5], Kazuhiko Matsuoka[1], Daisuke Tsuji[1,5], Ikuo Kawashima[2,5], Hiromi Akeboshi[3,5], Yasunori Chiba[3,5], Yoshifumi Jigami[3], Hitoshi Sakuraba[4,5].** [1]University of Tokushima, Tokushima, Japan; [2]Tokyo Metropolitan. Institute of Medical Science, Metropolitan Organization for Medical Research, Tokyo, Japan; [3]National Institute of Advanced Industrial Science and Technology, Tsukuba, Japan; [4]Meiji College of Pharmaceutical Sciences, Tokyo, Japan; [5]NIBIO, Osaka, Japan.

2:50 pm–3:10 pm 197 Down regulation of galectin-3 expression in prostate adenocarcinoma is caused by its promoter hypermethylation: Development and validation of a methylated marker for early diagnosis of prostate cancer. **Hafiz Ahmed[1], Francesco Cappello[2], Vito Rodolico[2], Gerardo R. Vasta[1].** [1]University of Maryland Biotechnology Institute, Baltimore, MD, USA; [2]University of Palermo, Palermo, Italy.

3:10 pm–3:30 pm 198 Translational aspects of Lactosylceramide Metabolism: mitigation of neovascularization and restensosis following balloon angioplasty. **Subroto Chatterjee, Ambarish Pandey, Antonina Kolmakova.** Johns Hopkins University, School of Medicine, Baltimore, MD, USA.

3:30 pm–3:50 pm 199 Modulating Glycosphingolipid Synthesis for the Treatment of Type 2 Diabetes and Fatty Liver Disease. **Nelson S. Yew[1], Hongmei Zhao[1], Malgorzata Przybylska[1], I-Huan Wu[1], Jinhua Zhang[1], Craig Siegel[1], Cynthia Arbeeny[1], James A. Shayman[2], Canwen Jiang[1], Seng H. Cheng[1].** [1]Genzyme Corporation, Framingham, MA, USA; [2]University of Michigan, Ann Arbor, MI, USA.

3:50 pm–4:10 pm 40 Regulation and pathophysiological implications of UDP-GlcNAc 2-epimerase/ManNAc kinase (GNE) as the key enzyme of sialic acid biosynthesis. **Werner Reutter[1], Stefan O. Reinke[2], Stephan Hinderlich[2].** [1]Charité-Universitaetsmedizin Berlin, Campus Benjamin Franklin, Institute of Biochemistry & Molecular Biology, Berlin, Germany; [2]Beuth Hochschule für Technik Berlin, Faculty Life Sciences and Technology, Laboratory of Biochemistry, Berlin, Germany.

4:10 pm–4:15 pm 201 Functional alteration of human β-hexosaminidase B for enzyme replacement therapy for GM2 gangliosidoses. **Kazuhiko Matsuoka[1,3], Tomomi Tamura[1,3], Daisuke Tsuji[1,3], Hitoshi Sakuraba[2,3], Kohji Itoh[1,3].** [1]Department of Medicinal Biotech, Institute for Medicinal Research, Graduate School of Pharmaceutical Sciences, The University of Tokushima, Tokushima, Japan; [2]Department of Analytical Chemistry, Meiji College of Pharmaceutical Sciences, Tokyo, Japan; [3]NIBIO, Japan.

4:15 pm–4:30 pm Coffee/Tea Break – *Foyer*

Poster Session III
Thursday, 4:30 pm–6:30 pm

202 B1 Subtleties of substrate recognition by Calreticulin, a lectin chaperone. **Garima Gupta[1], Avadhesha Surolia[1,2].** [1]Molecular Biophysics Unit, Indian Institute of Science, Bangalore, India; [2]National Institute of Immunology, New Delhi, India.

203 B2 Immobilization of Unmodified Glycans on the Glass Slide to Construct Carbohydrate Microarrays. **Sung-jin Park, Injae Shin.** Yonsei University, Seoul, Korea.

204 B3 Measurements of Glycosyltransferase Activities Using Glycan Arrays. **Sung-jin Park, Sung-kyun Ko, Injae Shin.** Yonsei University, Seoul, Korea.

205 B4 Superparamagnetic nanoparticles as platforms for studying protein-carbohydrate interactions. **Beatriz Pelaz[1,2], María Moros[1,2], Valeria Grazú[1,2], Jesús M. de la Fuente[1,2].** [1]Instituto Universitario de Investigación en Nanociencia de Aragón (INA), [2]University of Zaragoza, Zaragoza, Spain.

206 B5 Redesigning the carbohydrate recognition site of hen lysozyme. **Gabriel Gutierrez-Magdaleno, Enrique Garcia-Hernandez.** National Autonomus University of Mexico, Mexico City, Mexico.

207 B6 Nature of Lectin-carbohydrate Interactions and how to treat them by Computational Chemistry Tools. **Jaroslav Koca, Sushil K. Mishra, Jan Adam, Zdenek Kriz, Michaela Wimmerova.** National Centre for Biomolecular Research, Faculty of Science, Masaryk University, Brno, Czech Republic.

208 B7 Glycoconjugates (GC) in discrimination of GC-recognition systems of probiotic microorganisms. New potential keys for strains and their glycometabolome typing. **Vladimir M. Lakhtin, Vladimir A. Alyoshkin, Mikhail V. Lakhtin, Stanislav S. Afanasyev.** G.N. Gabrichevsky Institute of Epidemiology and Microbiology, Moscow, Russia.

209 B8 Carbohydrate-sensitive proteins in study of glycoenzymes. **Vladimir M. Lakhtin[1], Mikhail V. Lakhtin[1], Nikolay V. Bovin[2].** [1]Gabrichevsky Institute of Epidemiology & Microbiology, Moscow, Russia; [2]Shemyakin and Ovchinnikov Institute of Bioorganic Chemistry, Moscow, Russia.

210 B9 High-specificity affinity reagents for *N*-glycosylation site mapping and glycomics. **Loretta Yang[1], Khanita Kharaveg[2], Elisa Fadda[1], Nina E. Weisser[1], Kelley W. Moremen[2], Robert J. Woods[1,2].** [1]School of Chemistry, National University of Ireland, Galway, Galway, Ireland; [2]Complex Carbohydrate Research Center, University of Georgia, Athens, GA, USA.

211 B10 Combining Computational Carbohydrate Threading with Glycan Array Data to Define the 3D Epitope of an Anti-tumor Antibody. **Robert J. Woods[1,2], Matthew B. Tessier[1], Kate Rittenhouse-Olson[3], Jamie Heimburg[3], Andrew M. Gulick[4].** [1]Complex Carbohydrate Research Center, University of Georgia, Athens, GA, USA; [2]School of Chemistry, National University of Ireland, Galway, Galway, Ireland; [3]Department of Biotechnical and Clinical Laboratory Sciences, State University of New York, Buffalo, NY, USA; [4]Hauptman-Woodward Institute, Department of Structural Biology, State University of New York, Buffalo, NY, USA.

212 B11 Glycan Receptor-Binding Specificity in Human Adaptation of Influenza A Virus Hemagglutinin. **Karthik Viswanathan[1,2,3], Rahul Raman[1,2,3], Zachary Shriver[1,2,3], S Raguram[1,2,3], V Sasisekharan[1,2,3], Ram Sasisekharan[1,2,3].** [1]Department of Biological Engineering, Massachusetts Institute of Technology, Cambridge, MA, USA; [2]Harvard-MIT Division of Health Sciences and Technology, Massachusetts Institute of Technology, Cambridge, MA, USA; [3]Koch Institute of Integrative Cancer Research, Massachusetts Institute of Technology, Cambridge, MA, USA.

213 B12 ST3Gal.I sialyltransferase relevance in bladder cancer tissues and cell lines. **Paula A. Videira[1], Manuela Correia[1], Nadia Malagolini[2], Hélio J. Crespo[1], Dário Ligeiro[3], Paulo Severino[1], Hélder Trindade[1,3], Fabio Dall'Olio[2].** [1]CEDOC, Faculdade de Ciências Médicas, FCM, Universidade Nova de Lisboa, Lisboa, Portugal; [2]Department of Experimental Pathology, University of Bologna, Via S. Giacomo 14, 40126; [3]Centro de Histocompatibilidade do Sul, Alameda das Linhas de Torres, Lisboa, Portugal.

214 B13 N-Glycosylation of eFactor VIIIc is Reguired for Endothelial Cell Proliferation and Matrigel™ Invasion. **Krishna Baksi[1], Subiman Saha[2], Aditi Banerjee[2], Dipak K. Banerjee[2].** [1]Department of Anatomy and Cell Biology, School of Medicine, Universidad Central del Caribe, Bayamón, PR; [2]Department of Biochemistry, School of Medicine, University of Puerto Rico, San Juan, PR, USA.

145 B14 Purification of glycophorin C and chracterization of its N - glycosidic chain - receptor for Plasmodium falciparum antigen EBA – 140. **Ewa Jaskiewicz[1], Maria Duk[1], David Ashline[2], Elwira Lisowska[1].** [1]Institute of Immunology and Experimental Therapy, Wroclaw, Poland; [2]The Glycomics Center, University of New Hampshire, Durham, NH, USA.

216 B15 Role of E-cadherin N-glycosylation profile in a mammary tumor model. **Hugo Osorio[1], Salome S. Pinho[1,2], Mihai Nita-Lazar[3], Joana Gomes[1], Celia Lopes[2], Fatima Gartner[1,2], Celso A. Reis[1,4].** [1]Institute of Molecular Pathology and Immunology of the University of Porto (IPATIMUP), Rua Dr Roberto Frias s/n, Porto, Portugal; [2]Institute of Biomedical Sciences of Abel Salazar (ICBAS), University of Porto, Largo Prof. Abel Salazar, Porto, Portugal; [3]Boston University Medical Center, Boston, MA, USA; [4]Medical Faculty, University of Porto, Alameda Prof. Hernâni Monteiro, Porto, Portugal.

217 B16 Up-regulation of plasma membrane-associated sialidase augments malignant properties of cancer cells through activation of EGFR signaling. **Taeko Miyagi, Keiko Hata, Kazuhiro Shiozaki, Kazunori Yamaguchi, Tadashi Wada.** Miyagi Cancer Center Research Institute, Natori, Japan.

218 B17 Suppression of FUT4 down-regulates the expression and activation of PKCa and increases apoptosis of A431 cells. **Xuesong Yang, Shuang Jia, Zhengmei Zhu, Qiu Yan.** Department of Biochemistry and Molecular Biology, Dalian Medical University, Liaoning Provincial Core Laboratory of Glycobiology and Glycoengineering, Dalian, People’s Republic of China.

219 B18 Cytoplasmic expression of GlcA-GlcNH$_3^+$ on heparan sulfate closely correlates with malignancy of breast cancer. **Masahiro Fujii[1,2,3], Akiko Yusa[2], Yukihiro Yokoyama[3], Toshio Kokuryo[3], Masato Nagino[3], Hiroji Iwata[4], Hirotoshi Utsunomiya[5], Takeshi Ishimaru[6], Mamoru Kyogashima[2,7], Reiji Kannagi[2]** [1]Department Breast Oncology, Aichi Cancer Center, Aichi

Hospital, Aichi, Japan; [2]Division of Molecular Pathology, Aichi Cancer Center Research Institute, Aichi, Japan; [3]Division of Surgical Oncology, Department of Surgery, Nagoya University Graduate School of Medecine, Nagoya, Japan; [4]Department of Breast Oncology, Aichi Cancer Center Hospital, Aichi, Japan; [5]Wakayama Medical University Department of Internal Medicine, Wakayama, Japan; [6]Seikagaku Biobusiness Corporation; [7]Department of Oncology, Nagoya City University Graduate School of Pharmaceutical Science, Nagoya, Japan.

220 B19 Imaging technology of sialo-glycoconjugate molecular species by a combination of TLC-Blot and MALDI-TOF. **Tania Valdes-Gonzalez[1], Naoko Goto-Inoue[2], Takahiro Hayasaka[2], Mitsutoshi Setou[2], Hironobu Ishiyama[1], Takao Taki[3].** [1]Third Institute of Drug Discovery, Otsuka Pharmaceutical Co. Ltd., Tokushima, Japan; [2]Department of Molecular Anatomy, Molecular Imaging Frontier Research Center, Hamamatsu University School of Medicine, Japan; [3]Institute of Biomedical Innovation, Otsuka Pharmaceutical Co. Ltd., Tokushima, Japan.

221 B20 Chemical characterization of oligosaccharides in chimpanzee, bonobo, gorilla, orangutan, and siamang milk or colostrum. **Tadasu Urashima[1], Akitsugu Senda[1], Michael Messer[2], Olav T. Oftedal[3].** [1]Obihiro University of Agricultural & Veterinary Medicine, Obihiro, Hokkaido, Japan; [2]The University of Sydney, NSW, Australia; [3]Smithsonian Environmental Research Center, Edgewater, MD, USA.

222 B21 Molecular mechanism for differential recognition of the C-5 substituents of sialic acid by CMP-sialic acid synthetases: Identification of the evolutionally conserved sialic acid recognition region. **Akiko Fujita[1,2,4], Chihiro Sato[1,2,3], Yuko Yasukawa[1], Anja K. Münster-Kühnel[5], Rita Gerardy-Schahn[5], Ken Kitajima[1,2,3].** [1]Graduate School of Bioagricultural Sciemce, Nagoya University, Nagoya, Japan; [2]Bioscience & Biotechnology Center, Nagoya University, Nagoya, Japan; [3]JST-CREST, Japan; [4]Global COE Systems Biology, Japan; [5]Medical Hochsch. Hannover, Hannover, Germany.

223 B22 Insights Into Evolutionary History Of Animal Sialyltransferases.
Anne Harduin-Lepers[1], Jean-Michel Petit[2], Rosella Mollicone[3], Philippe Delannoy[1], Rafael Rafael[3], Daniel Daniel[2]. [1]Laboratoire de Glycobiologie Structurale et Fonctionnelle, CNRS, UMR 8576, Université des Sciences et Technologies de Lille, Villeneuve d'Ascq, France; [2]Laboratoire de Génétique Moléculaire Animale, INRA UMR 1061, Université de Limoges Faculté des Sciences et Techniques, Limoges, France; [3]Unité de Microenvironnement et physiologie de la différenciation, INSERM U602, Université de Paris Sud XI, Villejuif, France.

116 B23 Syndecan-4 regulates the cell-surface trafficking and activity of pro-fibrotic factor Transglutaminase-2. **Alessandra Scarpellini[1], Hugues Lortat-Jacob[2], Timothy Johnson[3], Elisabetta AM. Verderio[1].** [1]Nottingham Trent University, Nottingham, UK; [2]Institut de Biologie Structurale CNRS-CEA-UJF, Grenoble, France; [3]Academic Nephrology Unit, Medical School, University of Sheffield, Sheffield, UK.

173 B24 Immunological disorder analysis of polylactosmaine synthase-deficient mice.
Akira Togayachi[1], Yuzuru Ikehara[1], Hiroyasu Ishida[1,2], Yuko Kozono[1], Hideki Matsuzaki[1], Takashi Sato[1], Takashi Kudo[3], Satoru Takahashi[3], Jun Hirabayashi[1], Hisashi Narimatsu[1]. [1]Research Center for Medical Glycoscience (RCMG), AIST, Ibaraki, Japan; [2]Institute of Clinical Medicine, University of Tsukuba, Ibaraki, Japan; [3]Institute of Basic Medical Sciences, University of Tsukuba, Ibaraki, Japan.

180 B25 Role of N-acetylglucosaminyltransferase III and V in the Post-Translational Modifications of E-cadherin. **Salomé S. Pinho[1,2], Celso A. Reis[1,3], Joana Paredes[1], Ana Maria Magalhães[1], António Carlos Ferreira[1], Joana Figueiredo[1], Wen Xiaogang[1,3], Fátima Carneiro[1,3], Fátima Gartner[1,2], Raquel Seruca[1,3].** [1]Institute of Molecular Pathology and Immunology of the University of Porto (IPATIMUP), Portugal; [2]Institute of Biomedical Sciences of Abel Salazar (ICBAS), Portugal; [3]Medical Faculty, University of Porto, Portugal.

187 B26 Glycosylation of voltage-gated potassium channels affects neuronal expression and localization. **Desiree A. Thayer, Lily Y. Jan.** University of California, San Francisco, CA, USA.

194 B27 Novel aspects of the catalytic mechanism of human blood group B galactosyltransferase from NMR and microcalorimetry. **Nora Sindhuwinata[1], Hannelore Peters[1], Thies Koehli[1], Eva Munoz[1,2], Thomas Weimar[1], Monica M. Palcic[3], Thomas Peters[1].** [1]University of Luebeck, Institute of Chemistry, Luebeck, Germany; [2]University of Santiago de Compostela, Compostela, Spain; [3]Carlsberg Laboratory, Valby, Denmark.

201 B28 Functional alteration of human β-hexosaminidase B for enzyme replacement therapy for GM2 gangliosidoses. **Kazuhiko Matsuoka[1,3], Tomomi Tamura[1,3], Daisuke Tsuji[1,3], Hitoshi Sakuraba[2,3], Kohji Itoh[1,3].** [1]Department of Medicinal Biotech, Institute for Medicinal Research, Graduate School of Pharmaceutical Sciences, The University of Tokushima, Tokushima, Japan; [2]Department of Analytical Chemistry, Meiji College of Pharmaceutical Sciences, Tokyo, Japan; [3]NIBIO, Japan.

230 (LB-3) B29 Glycosylation pathways of human prostate cancer cells**. Yin Gao, Inka Brockhausen.** Department of Biochemistry, Queen’s University, Kingston, Ontario, Canada.

232 (LB-5) B30 Glycosylated peptide random bead libraries for biomarker discovery. **Steven B.. Levery, Stjepan K. Kracun, Emiliano Clo, Henrik Clausen, Knud J. Jensen, Ola Blixt.** University of Copenhagen, Copenhagen, Denmark.

233 (LB-6) B31 Synthesis and applications of artificial siderophores with sucrose backbone. **Marta M. Andrade, Rui C. Pinto**. Requimte, DQ, FCT, Universidade Nova de Lisboa, Portugal.

235 (LB-8) B32 A convenient method for synthesis of glyco-nanoparticles/beads for lycan function analysis. **Xichun Zhou[1], Nick Knowlton[1], Yen-Jun Chuang[2], Zhengwei Pan[3].** [1]ADA Technologies, Inc., Littleton, CO, USA, [2]Faculty of Engineering, University of Georgia, Athens, GA, USA, [3]Department of Physics and Astronomy, University of Georgia, Athens, GA, USA

237 (LB-10) B33 A Bivalve D-Galactose-binding Lectin Transduced the Growth Static Signal for Burkitt's Lymphoma Cells Mediated to Gb3 Glycosphingolipid. **Ryo Matsumoto[1], Sarkar M.A. Kawsar[1], Yuki Fujii[1], Hidetaro Yasumitsu[1], Masahiro Hosono[2], Kazuo Nitta[2], Yasuhiro Ozeki[1]**. [1]Yokohama City University, Yokohama, Japan, [2]Institute of Molecular Biomembrane & Glycobiology, Tohoku Pharmaceutical University of Sendai, Sendai, Japan

238 (LB-11) B34 Frontal Affinity Chromatography for Glycan Binding Profiling of alectin-1 Purified from American Bullfrog (Rana catesbeiana) Oocyte. **Sarkar M.A.. Kawsar, Ryo Matsumoto, Yuki Fujii, Hidetaro Yasumitsu, Yasuhiro Ozeki.**Yokohama City University, Yokohama, Japan.

240 (LB-13) B35 Human Intestinal Starch Digestion Enzymes Maltase-glucoamylase and Sucrase-Isomaltase. **David R. Rose[1,2], Lyann Sim[2], Buford L. Nichols[3], Roberto Quezada-Calvillo[4], Bruce Hamaker[5], Mario Pinto[6]**. [1]University of Waterloo, Ontario, Canada, [2]University of Toronto, Ontario, Canada, [3]Baylor College of Medicine, Houston, TX, [4]University of San Luis Potosi, Mexico, [5]Purdue University, West Lafayette, IN, [6]Simon Fraser University, BC, Canada.

242 (LB-14) B36 A Potent Glycomimetic Selectin Antagonist (GMI-1070) in Clinical Trials for Sickle Cell Disease. **John Patton[1], Arun Sarkar[1], Beatrice Wagner[2], Paul Frenette[3], Helen hackray[1], Beat Ernst[2], Scott Simon[4], John L. Magnani[1]**. [1]GlycoMimetics Inc., Gaithersburg, MD, USA, [2]University of Basel, Basel Switzerland, [3]Mt. Sinai School of Medicine, New York, NY, University of California, Davis CA, USA.

244 (LB-16) B37 Calculating Theoretical Binding Energies for Hemagglutinin-Host Cell Receptor Interactions – Computational Prediction of Hemagglutinin Specificity. **Jodi A. Hadden[1], B. Lachele Foley[1], Loretta Yang[2], John P. Nolan[3], S. Mark Tompkins[1], Robert J. Woods[1,2]**. [1]University of Georgia, Athens, GA, USA; [2]National University of Ireland, Galway, Ireland; La Jolla Bioengineering Institute, La Jolla, CA, USA

245 (LB-18) B38 Developing insights into the *O*-GlcNAc transferase substrate peptide; sequon identity from dissecting the results of a peptide library screen. **David E. Blair[1, 2], Xiaowei Zheng[1], Shalini Pathak[1] Julie Frearson[2] and Daan M.F. van Aalten[1]**. [1]Division of Molecular Microbiology, [2]Division of Biological Chemistry and Drug Discovery, College of Life Sciences, University of Dundee, Scotland, UK

246 (LB-19) B39 The GT2 enzyme NodC as a model for studying processive chitin synthase. **Helge C. Dorfmueller and Daan M.F. van Aalten**. Division of Molecular Microbiology, College of Life Sciences, University of Dundee, Scotland, UK

Friday, December 4, 2009

8:30 am–10:00 am **Registration**

7:00 am–8:30 am **Breakfast –** ***San Cristobal Ball Room***

Plenary Lecture VII
Friday, 8:45 am–9:30 am

Location: **San Geronimo Ball Room – A, B & C**
Chair: **Sandro Sonnino,** Università degli Studi di Milano, Italy

224 Jin-ichi Inokuchi.
Institute of Molecular Biomembranes and Glycobiology, Tohoku Pharmaceutical University, Sendai, Japan

Inhibition of Ganglioside Biosynthesis as a Novel Therapeutic Approach in Insulin Resistance

Plenary Lecture VIII
Friday, 9:35 am – 10:20 am

Location: **San Geronimo Ball Room – A, B & C**
Chair: **Avadesha Surolia,** National Institute of Immunology, India

225 Jean-Claude Michalski
Université de Lille1, Structural and Functional Glycobiology Unit CNRS, Lille, France

Link between quality control and ERAD process of newly synthsized glycoproteins.

10:20 am–10:50 am **Coffee/Tea Break** – ***Foyer***

Plenary Lecture IX
Friday, 10:50 am–11:35 am

Location: **San Geronimo Ball Room – A, B & C**
Chair: **Richard D. Cummings,** Emory University, USA

226 Patrick J. Brennan
Department of Microbiology, Immunology and Pathology, Colorado State University, Fort Collins, CO, USA

Towards Complete Molecular Definition of the Cell Wall of Mycobacterium tuberculosis and Mycobacterium leprae.

11:40 am **Closing Ceremony and Invitation to GLYCO XXI**

12:15 pm **Farewell Drink**

Abstracts

Program/Abstract# 1

From milk oligosaccharides to N-linked sugar chains: our forty years journey in glycobiology

Akira Kobata
The Noguchi Institute, Japan

Human milk contains a various oligosaccharides. By applying a fingerprinting method for the analysis of individual milk samples, several oligosaccharides were found to be missing in the milk samples collected from non-secretor or Lewis negative mothers. This finding opened a way to elucidate the enzymatic basis of ABO and Lewis blood types in humans. Based on this success, a strategy appropriate to investigate the structures and functions of the N-linked sugar chains of glycoproteins was devised. N-Linked sugar chains were released quantitatively as oligosaccharides by enzymatic or chemical treatment of a glycoprotein, and labeled by NaB^3H_4 reduction. After fractionation by gel-permeation chromatography and lectin-affinity chromatography, structure of each radioactive oligosaccharide was determined by a series of sensitive methods developed in our laboratory. Oligosaccharide patterns of various glycoproteins obtained by using these techniques demonstrated the occurrence of structural rules of the N-linked sugar chains, and site-specific, organ-specific and species-specific N-glycosylation of proteins, which served as important bases for the later development of glycobiology. Furthermore, these methods enabled us to demonstrate the structural alteration of the sugar chains of a glycoprotein induced by disease state of the producing cells, such as rheumatoid arthritis and malignancy. Studies of glycoproteins in the brain nervous system through aging revealed that many altered glycosylation is induced by aging. Accordingly, glycobiology has expanded into fields such as glycopathology and glycogerontology.

Program/Abstract# 2

The importance of unambiguous glycan structures

Johannes F.G. Vliegenthart
Bijvoet Center Biomolecular Research, Utrecht University, Utrecht, The Netherlands

At the 2nd International Symposium on Glycoconjugates (Lille, France, 1973) we described the parameters needed to define glycan structures as well as how to determine these by mass spectrometry and NMR spectroscopy. In 1977, at the 4th Symposium (Woods Hole, USA), we could present the unambiguous results for a large number of individual glycans. The relevance of correct structures followed directly from the construction of metabolic pathways and enzyme specificities.

For the future the emphasis will be on the role of glycans in their natural environment. The function of glycans in biological systems is usually mediated via interaction and recognition processes with complementary biomolecules. The features of (micro)heterogeneity and multivalency are complicating factors in studies to pinpoint the contribution of glycans to overall effects. Further sophistication of methodology is necessary to gain insight in the interplay complex molecules in larger entities. The dynamics of these systems constitute special problems to solve.

Program/Abstract# 3

Studies on enzymatic formation and degradation of glycoproteins

William J. Lennarz
Department of Biochemistry and Cell Biology, Stony Brook University, Stony Brook, New York, USA

The objective of this talk is to summarize my current understanding of the enzyme complex that *N*-glycosylates proteins and the set of proteins that is responsible for the destruction of mis-or unfolded glycoproteins (and proteins). The initial stages of the *N*-glycosylation process is assembly of the Dol linked precursor Man-P-Dol and also Glc-P-Dol, which is used in a later stage of the assembly process. This process utilizes the respective sugar nucleotide precursors, and Dol-P as acceptors. Also involved is UDP-GlcNAc serving as donor of GlcNAcP, and in a second stage, donor of GlcNAc to form $Dol\text{-}PP(GlcNAc)_2$. In this assembly in a process still not totally understood molecular flip flops occur. Finally, the oligosaccharide chain is transferred for the lipid carrier to Asn-X-Ser/Thr- sites in the nascent polypeptide chain. This process may occur while oligosaccharyl transferase (OT) exists in a complex with Sec61 and ribosomes, Dr. Yoichiro Harada will present

our studies on this trimeric complex. As reported by others, an error in translation often leads to the inability of both membrane proteins or secretory proteins to correctly fold. Cells have evolved a mechanism to dispose of these misfolded proteins. This involves translocation of the misfolded proteins to the cytosol where they can be degraded by the proteasome. An important protein in the translocation process is cdc48 (p97 in mammals). This protein apparently provides the driving force to move misfolded proteins out of the ER to the cytosol. As to be reported by Dr. Li, mutational studies of amino acids residues have revealed the importance of the C-terminus of cdc48.

These studies have been supported by NIH grants GM33184 and GM33185.

Program/Abstract# 4

Protein glycosylation, conserved from yeast to man

Widmar Tanner
Univ. of Regensburg, 93040 Regensburg, Germany

During the exciting times of molecular biology within the past 50 years, proteins and nucleic acids have dominated the field. Carbohydrates (besides their role in energy metabolism) had remained in the shade. Since about 20 years there exists an increasing awareness, however, that carbohydrates, as information molecules par excellence, play important roles especially in regulating the development of higher organisms. Decisive progress in understanding the biochemistry and molecular biology of glycoproteins has been made due to work with model organisms like yeast. It started with uncovering the role of dolichol-activated sugars, proceeded to the elucidation of the genes involved in protein N-glycosylation and O-mannosylation and will certainly not end with the biochemical studies of multi-enzyme complexes and investigations, which helped to identify various types of human congenital disorders of glycosylation (CDGs). The latter is true for N-linked and O-linked saccharides. On the other hand, since yeast is a unicellular organism, the pathways and reactions conserved from yeast to man must play cellular, most likely intracellular functional roles and yeast may be helpful as well in elucidating the detailed molecular reasons for certain congenital disorders.

The better we understand the different roles of oligo- and polysaccharides which modify proteins and lipids, the more pronounced their contribution will be to successfully tackle medical and pharmaceutical challenges in the future. Thus the occupation with the chemistry and biology of carbohydrates and glycoconjugates promises fascinating science, the solution of fundamental biological questions, and eventually the successful transfer of the knowledge to applied fields.

Program/Abstract# 5

The past and new challenges of sialic acid research

Roland Schauer
University of Kiel, Institute of Biochemistry, Germany

Sialic acids (Sia) are monosaccharides with multiple chemical and biological properties. They were first isolated about 70 years ago, but it took scientists all over the world many years to elucidate their correct structures and to investigate their metabolism and functions. Although we now are knowledgable about their occurrence in microorganisms, insects and higher animals, their chemical diversity, linkage to numerous glycoproteins, gangliosides and polysaccharides, their metabolism and the genes involved, their participation in cell biological and pathological events, as well as their pharmaceutical application, many questions are still open. Most of the functions of Sia can be described as either masking biological recognition sites or representing recognition epitopes (ligands for receptors). In this way Sia render cells as "self", influence immune reactions, regulate apoptosis or affect receptor function, growth, differentiation and ageing. Among the new challenges are, for example, further elucidation of the roles of polysialic acids, which influence differentiation and function of neuronal tissues. The properties of various Sia can be exploited by microorganisms, in particular viruses, and by tumor cells, and as Sia are involved in many infectious, immunological, malignant, psychiatric and degenerative diseases, work in these areas will be necessary. A better understanding of the regulation of Sia biosynthesis and degradation by, for example, hormones and other substances, the mechanism of sialic acid-receptor interactions, and knowledge of the supramolecular arrangement of sialylated glycans in cell membranes, will help in finding cures for these diseases.

Program/Abstract# 6

Use of Glycomics to target therapeutic enzymes

Roscoe O. Brady
National Institutes of Health, Bethesda, MD 20892

Enzyme replacement therapy (ERT) for metabolic storage disorders requires effective delivery of the enzyme to metabolite-storing cells. Targeting of glucocerebrosidase that is deficient in patients with Gaucher disease is necessary. Glucocerebrosidase isolated from human placental tissue reduced accumulating glucocerebroside in the liver and circulation of patients. Targeting that glucocerebrosidase to lipid-storing macrophages resulted from an association of phosphatidylserine with the enzyme. Because it was not possible to scale-up the purification procedure, a

method based on hydrophobic column chromatography was developed. Glucocerebrosidase obtained in this fashion was mainly taken up by hepatocytes and had minimal effect on accumulated glucocerebroside. Lysosomal enzymes such as glucocerebrosidase are glycoproteins. Macophages have a lectin on their surface that reacts avidly with mannose-terminal glycoproteins. Synthetic glycosylation of placental glucocerebrosidase with triantennary trimannosyldilysine provided a 4-fold increase in the delivery of enzyme to macrophages (*J Biol Chem* 1982; 257:2193). A 50-fold increase was obtained through the use of 3 exoglycosidases to remove sialic acid, galactose and N-acetylglucosamine residues forming mannose-terminal gluocerebrosidase (*Biochim Biophys Acta* 1981; 673:425). This modification provided the first clinical benefit from ERT to a patient with a metabolic storage disorder (*Proc Natl Acad Sci USA* 1990; 87; 1913). More than 5,500 patients with Gaucher disease are now receiving this treatment. Targeting therapeutic enzymes in patients with other metabolic storage disorders will be discussed.

Program/Abstract# 7

Mass spectrometric strategies for glycomics and glycoproteomics

Anne Dell
Imperial College London

Ultra-high sensitivity mass spectrometric strategies incorporating MALDI-MS/MS and nano-electrospray(ES)-MS/MS enable very complex mixtures of glycans and glycopeptides from biological extracts of cells and tissues to be screened thereby revealing the types of glycans present and, importantly, providing clues to structures that are likely to be functionally important (Ref 1). Data emerging from our glycomic and glycoproteomic programmes of collaborative research, which are helping to provide new insights into the functions of glycans in biological systems, will be described.

Our glycomic methodologies are being exploited by the NIH Consortium for Functional Glycomics whose Analytical Core, located at Imperial College, is carrying out high throughput analyses of murine and human haematopoietic cell populations in order to provide a glycomics data resource for the glycobiology community. Information emerging from this programme will be highlighted together with progress on the development of informatic tools to manage the large volumes of data being acquired.

1. Tissot B, North SJ, Ceroni A, Pang PC, Panico M, Rosati F, Capone A, Haslam SM, Dell A, Morris HR. (2009 "Glycoproteomics: past, present and future." FEBS Lett. 583(11):1728–35.

Program/Abstract# 8

Identifying and exploiting the CIS and trans ligands of CD22

James C. Paulson, Weihsu (Claire) Chen, Gladys Completo, Corwin Nycholat, Mary E. O'Reilly, Cory Rillahan, Ramya T. N. C., Hua Tian, Ying Zeng
Department of Chemical Physiology, The Scripps Research Institute, La Jolla, CA, 92037, USA

Glycans decorate the surface of cells with unique moleculular signatures that play critical roles in the communication of cells. The information in this sugar 'code' is decoded by glycan binding proteins that mediate cellular responses. Our laboratory is interested in understanding the roles of glycoprotein ligands of CD22, a negative regulator of B cell receptor signaling. CD22 is a member of the siglec (Sialic acid-binding immunoglobulin-like lectin) family, and recognizes glycoprotein ligands that contain glycans with the sequence, NeuAcα2-6Gal. The activity of CD22 is modulated by its ligands expressed both on B cells (cis ligands), and other cells contacted by B cells (trans ligands). To understand the role of ligands in CD22 function we have taken a two-pronged approach. To identify the cis and trans ligands of CD22 we have initiated a proteomics-scale study of sialylated glycoproteins on B cells and other cells to identify both the total 'sialoglycoproteome' and the ligands of CD22. In a separate approach we have designed synthetic glycan ligands that compete with the natural ligands and serve as probes of the CD22 ligand-binding site. These probes prove to be an efficient way of delivering agents to B cells, and have both helped understand the biological role of CD22 and suggest approaches for ligand based approaches for B cell depletion therapy in B cell leukemias and autoimmune disease. (Funded in part by NIH grants GM60938 and AI16165).

Program/Abstract# 9

Development of Glyco-cancer biomarkers using novel technologies

Hisashi Narimatsu
Research Center for Medical Glycoscience, AIST

Detection of cancer at the early stage for curable surgical operation is the most difficult task. Our basic concept is that glycoproteins produced by cancer cells alter in their glycan structures even though the proteins are common, ubiquitous, abundant and familiar. To discover glycoproteins produced by cancer cells, which occupied probably less than 1% of the normal tissue in the relevant organ at the early stage, we first analyzed supernatants of cultured cancer cells using three technologies. I) Quantitative real

time PCR for glycogenes in order to expect the glycan structures of secreted glycoproteins. II) Analysis by Lectin micro array for culture supernatants to select lectins which distinguish secreted glycoproteins. III) IGOT method to determine carrier proteins having the specific lectin epitope in a high-throughput manner. We identified many glycoproteins of which glycan structures altered prodused by cancer cells. These candidate glcoproteins were immunoprecipitated from serum using commercially available antibodies, and their glycan alteration was examined by Lectin microarray, and finally determined by masspectrometry technology.

Program/Abstract# 10

Glycomics analysis combined with a genome-wide association study identifies loci involved in regulation of the human plasma N-glycome

P. M. Rudd[1], C. Hayward[4], A. Essafi[4], J. E. Huffman[4], A. Knezevic[5], O. Polasek[3,6], O. Gornik[5,7], V. Vitart[4], L. Zgaga[6], M. Pucic[5], I. Redzic[5], F. Borovecki[8], N.D. Hastie[4], J.F. Wilson[1], A.F. Wright[4], H. Campbell[1,4], G. Lauc[5,7], I. Rudan[9,2,3]
[1]NIBRT,Univ. College Dublin, Ireland, [2]Croatian Centre for Global Health, Univ. of Split, Croatia, [3]Gen-Info Ltd, Croatia, [4]MRC Human Genetics Unit, Edinburgh, UK, [5]University of Zagreb, Croatia, [6]Univ. of Zagreb Medical School, Croatia, [7]Glycobiology Laboratory, Genos Ltd, Croatia, [8]Centre for Functional Genomics, Univ. of Zagreb, Croatia, [9]Public Health Sciences, The Univ. of Edinburgh, UK

The human glycome is several orders of magnitude more complex than the human proteome. Genetic defects that affect protein glycosylation cause >30 human diseases. Population variability in terminal glycans contributes to protein heterogeneity and can be advantageous for evading pathogens and adapting to changing environments. Our new robotic high-throughput analytical methods allow us to combine high-throughput glycomics with high-throughput genomics. HPLC was used to quantify levels of 16 groups of N-linked glycans on the human plasma proteins in about 2,800 island isolates. As proof of principle, we selected the bi-antennary N-linked glycan $GlcNAc_2Man_3GlcNAc_2$. Subsequent GWAS using 317,503 single nucleotide polymorphisms (SNPs) identified genetic loci influencing variation in this glycan. We identified FUT8 on chromosome 14 as the key regulator, reaching genome-wide significance in both discovery and replication cohorts. Meta-analysis showed that the SNP most strongly associated with levels of this glycan was rs11621121 (p=7.59x10-18). This is highly plausible, because glycan A2 is a known substrate for FUT8. Thus human plasma N-glycans are amenable to genome-wide association studies and their genetic regulation shows similar characteristics to other biological quantitative traits.

Program/Abstract# 11

Glycans to modulate dendritic cell mediated immune responses

Yvette van Kooyk, Ingegorg Streng-Ouwehand, Satwinder K. Singh, Manja Litjens, Wendy Unger
VUmc, Amsterdam, the Netherlands

Dendritic cells (DC) are specialized in the recognition of pathogens and play a pivotal role in the control of immunity. Yet DCs are also important for homeostatic control recognizing self antigens and tolerizing its environment, indicating that the nature of the antigen it recognizes may steer a DC towards immunity or tolerance. We have shown that the C-type lectins DC-SIGN and MGL are specific antigen uptake receptors that process antigen for presentation to T cells. Glycan modification of antigen with DC-SIGN or MGL binding glycans can strongly affect the antigen uptake and presentation capacity of DC. Our data demonstrate that the antigens are differentially routed intracellularly, leading to distinct CD4 and CD8 T cell responses.

Several C-type lectins have reported to be also signalling receptors, which modify cytokine responses, leading to specific T cell differentiation. Our goal is to instruct DC using glycan modified antigens compositions to tailored immune responses that may control immunity against cancer or inhibit inflammatory responses such as auto-immune diseases.

Program/Abstract# 12

The regulation of immunological tolerance and autoimmunity by 9-O-acetylation of sialic acid

Shiv Pillai, Ira Surolia, Stephan Pirnie, Annaiah Cariappa, Jesse Moya, Ajit Varki[2], Peter Gregersen, Kendra Taylor, Amy McQuay, Bruce Sands
Massachusetts General Hospital, Harvard Medical School Boston MA 02129

Sialic acid acetyl esterase is an enzyme that negatively regulates B lymphocyte antigen receptor signaling and is required for the maintenance of immunological tolerance as shown in studies on engineered mutant mice. Heterozygous or homozygous loss of function variants of *SIAE* were identified in Caucasian subjects with relatively common autoimmune disorders. The calculated Odds Ratio for *SIAE* mutations in autoimmune patients is 7.5. *SIAE* variants represent the first mechanistically confirmed genetic link to defective B cell

tolerance in relatively common human autoimmune disorders. The relevance of SIAE and of a 9-O-acetyl transferase to the regulation of humoral immune responses, immunological tolerance and autoimmunity will be discussed.

Program/Abstract# 13

9-O-acetylated sialoglycoproteins in visceral leishmaniasis: the multifaceted trigger modulating the erythrocyte biology

Angana Ghoshal[1], Sajal Samanta[1], Bibhuti Saha [2], Saulius Jarmalavicius[3], Peter Walden[3], Chitra Mandal[1]
[1]Infectious Disease and Immunology Division, Indian Institute of Chemical Biology, 4 Raja S.C. Mullick Road, Kolkata-700 032, India, [2]Department of Tropical Medicine, School of Tropical Medicine, Kolkata 700032, India, [3]Department of Dermatology, Charité-Universitätsmedizin Berlin, Humboldt University, 10098 Berlin, Germany

The exclusive presence of six distinct 9-*O*-acetyl sialoglycoproteins (9-*O*-AcSGPs) on erythrocytes of active VL patients (RBC_{VL}) and their involvement in triggering the alternate complement pathway has been reported. Concomitant high titers of anti-*O*-AcSGP-IgG_{VL} antibodies in patient's sera have also been demonstrated. This instigated us to explore the effect of sensitization of 9-*O*-AcSGPs on RBC_{VL} using anti-*O*-AcSGP-IgG_{VL} antibodies. Sensitized RBC_{VL} demonstrated altered morphology and membrane characteristics. Interestingly, sensitized aged mature RBC_{VL} evidenced higher oxidative stress as compared to the young erythrocytes and demonstrated features of apoptosis like phosphatidyl serine exposure which led to increased erythrophagocytosis, activation of caspase-3 and impairment of flippase activity. Additionally, the erythrocyte membrane integrity was unraveled by sequencing the newly induced 9-*O*-AcSGPs. Interestingly, one of these 9-*O*-AcSGPs, a 57 kDa sialoglycoprotein, was identified as fragment of eythrocytic alpha-I spectrin by MALDI-TOF-MS. The disparity in molecular weight of the 57 kDa, identified as alpha-I spectrin, instigated us to investigate the status of cytoskeletal proteins of RBC_{VL}. Accordingly, SDS-PAGE followed by mass spectrometry and Western blot analysis of purified spectrin of RBC_{VL} revealed the presence full length alpha and beta spectrin along with the 57 kDa band. Several biochemical methods demonstrated its purity, identity, sialylation and the presence of both *N*- and *O*-glycosylation. Interestingly, sensitized RBC_{VL}, showed an additional 130-kDa fragment and increased cytosolic calcium that triggered the activation of calpain I indicating the involvement of the 9-*O*-AcSGP. In contrast, all these alterations were not observed in RBC_N possibly due to complete absence of this sialoglycotope. Taken together, this is the first report encompassing the different facets of VL erythrocyte biology, where the main player is 9-*O*-AcSGP, which would probably help elucidate the basis for the anemia commonly associated with VL.

Program/Abstract# 14

Galectins in innate immunity: dual functions of host soluble β-galactoside binding lectins as damage-associated molecular patterns (DAMPs) and as receptors for pathogen-associated molecular patterns (PAMPs)

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The glycocalyx is found on the surfaces of host cells, microorganisms, and enveloped virus and is composed of various structurally different glycans that provide cell- or microorganism-specific "glycoinformation" that can be decoded by mammalian lectins. Example of such lectins are collectins, DC-SIGN and selectins, which, upon infection, trigger the innate immune response. These lectins can be considered as receptors for PAMPs (Pattern recognition receptors, PRRs) when they recognize non-self glycans presented by microorganisms. Most lectins are synthesized in the secretory pathway and readily presented on the cell surface, one notable exception being galectins, which are rather synthesized in the cytoplasm, segregated from their glycan ligands. Upon infection-initiated tissue damage and/or following prolonged infection, cytosolic galectins are either passively released by dying cells or actively secreted by inflammatory activated cells through a nonclassical secretory pathway. We have suggested that once exported, galectins can act as PRRs. Indeed, galectin-3 and -9 recognize a protozoa parasite, *Leishmania major*, while galectin-1 binds to HIV-1 in a β-galactoside-dependent manner, facilitating the infection. In addition, our recent data suggest that galectin-3 acts as immunomodulators in streptococcal pneumonia and leishmaniaosis. Galectin-3 activates and facilitates the local recruitment of neutrophils, dominant leukocytes at the frontline of the innate defense against infections. In addition to being implicated in pathogen recognition, galectins are dominantly found in lesions where pathogen-initiated tissue damage signals appear, this lectin family should thus also be considered as a damage-associated molecular patterns (DAMPs) candidate that orchestrates innate immune responses alongside the PAMPs system.

Program/Abstract# 15

Functional roles of mammalian structural units, ligand cluster and polyvalency in the Abrus precatorius agglutinin and glycoprotein recognition process

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Previous studies on one of the toxic type 2 ribosome inactivating proteins (RIP), *Abrus precatorius* agglutinin (APA),it was indicated that the recognition domains of APA were restricted mainly to monomer of Galβ1-3GalNAc ($\mathbf{T}_\alpha$, Thomsen-Friedenreich glycotope)>Galβ1-3/4GlcNAc (blood group precursor type **I/II**sequences) which are essential, but play very minor contribution in recognition process. In this study, APA recognition factors were expanded to include ligand clusters and polyvalent glycotopes by enzyme-linked lectinosorbent binding and inhibition assays. Based on the results of molar relative potency, the essential mammalian structural units are Galβ1-3GalNAcα/β1- ($\mathbf{T}_\alpha$/$\mathbf{T}_\beta$) > Galα1-4Gal (**E**) > Galβ1-3/4GlcNAc (**I/II**) and avidity for tri-, di-antennary $\mathbf{II}_\beta$, **T**, **E** and **II** monomerswere found to be 7.1×10^2, 4.0, 5.5, 3.7 and 2.4 times higher than monomeric Gal. When natural polyvalent glycotopes or clusters were tested and expressed by mass relative potency, high-density polyvalent $\mathbf{T}_\alpha$ and complex multivalent $\mathbf{I}_\beta$/$\mathbf{II}_\beta$ glycotopes greatly enhanced the affinity for APA over 10^4 times. Based on results available, it can be inferred that contribution of monomeric $\mathbf{T}_\alpha$, $\mathbf{II}_\beta$, $\mathbf{I}_\beta$, $\mathbf{E}_\beta$ and their clusters and polyvalency play critical roles in this recognition process. The binding intensities of these factors in decreasing order are: polyvalent $\mathbf{T}_\alpha$, $\mathbf{II}_\beta$/$\mathbf{I}_\beta$and $\mathbf{E}_\beta$>tri-antennary $\mathbf{II}_\beta$ >>monomeric $\mathbf{T}_\alpha$, $\mathbf{T}_\beta$, **I** and **II**>Gal >> GalNAc (weak). As one of type 2 RIP lectins, these recognition factors of B chain are likely to be crucial for attachment and endocytosis. A comparison of the differential recognition factors and combining sites of APA with those of other lectins (*Ricinus communis* agglutinin, RCA_1 and ricin) is also illustrated.

Program/Abstract# 16

Diversity of carbohydrate binding profile among D-galactoside binding lectins isolated from lower animals

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[Purpose] So many D-galactose- and/or D-galactoside-binding lectins are isolated from lower animals such as marine invertebrates and land amphibia, in addition to lectins obtained from plants and higher animals. However, we do not know why they recognize the same saccharide, though these lectins are seemed to function in the different contexts each other. To appear this point, we compare the carbohydrate-binding profile of D-galactoside binding lectins obtained from these lower animals by frontal affinity chromatography technology (FACT) with pyridilaminatide (PA)-oligosaccharides. [Methods] D-galactose-binding lectins were purified from mollusca, porifera, annelida, and amphibia using lactosyl-agarose. Each lectin conjugates into NHS-Sepharose gel. The affinity gel was packed into mini-column and set onto HPLC system. Twelve and fourty nine PA-oligosaccharides (10 pmol in 2 mL) were applied into the column at 0.25 mL /min and detected. FACT has done as follow. The extent of retardation volume (V-Vo) of each PA-oligosaccharide (V) from control PA-rhamnose (Vo) by the interaction through the column was detected by fluorescence (Ex. 310 nm/Em. 380 nm) and calculated. [Results] Six D-galactoside-binding lectins were purified from these creatures with a lactosyl-agarose affinity column and they appeared different molecular size each other. Glycan-binding profile of these D-galactoside-binidng lectins is classified as three types as follow: 1) complex type oligosaccharide with blanched type 2 lactosamine chains, 2) blood-type substances oligosaccharide, and 3) globotriose oligosaccharide using FACT analysis. [Conclusion] The FACT analysis suggests that these lectins known to be a D-galactoside-binding have more diversification on the glycan-binding properties than our recognition. By their diversified glycan-binding properties, they will be possible to be functioned the complex roles such as cellular regulation and innate immunity through the glycan-protein interaction.

Program/Abstract# 17

Galectin-1 and HIV virion interaction: how does galectin-1 (but not galectin-3) selectively facilitates HIV infection in CD4+ cells?

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HIV is known to carry tick layer of glycocalyx. Ones of the major glycans of this glycocalyx is attached to viral glycoprotein, gp120 coded by viral *env* gene. The first

infection step of HIV is interaction between gp120 and CD4 of susceptible cells. However this interaction is unexpectedly weak, therefore, it has been proposed that host molecules are exploited by HIV to facilitate its infection. Indeed, we previously reported that one of those host molecules is galectin-1 (1~3). Our published data indicate that galectin-1 increases HIV attachment to target cells in beta-galactoside binding-dependent manner and facilitates its infectivity. Thus, we hypothesized that galectin-1 directly cross-links the virions to $CD4^+$ cells. To test this possibility, we studied galectin-1 ligands expressed on virions and the target cells. Data obtained by lectin affinity chromatography showed that virus specifically bound to galectin-1 in beta-galactoside-dependent but mannose-independent manner. Consistent with the results that galectin-3 does not increase HIV infection, HIV-1 particles had a very weak affinity towards galectin-3. Since 90% of HIV-1 binding to galectin-1 was abolished when we used a virus without *env* gene. In addition, recombinant viral gp120 was found to bind strongly to galectin-1 and eluted with lactose but not with mannose, suggesting that the major ligands of HIV for galectin-1 is gp120. Further analyses are in progress to shed light on how galectin-1 but not galectin-3 recognizes gp120, and contributes HIV-1 pathogenesis in lymph nodes.

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This work has been supported by CIHR grant and C.S.P. hold a Canada Graduate Scholarship from CIHR.

Program/Abstract# 18

Synthetic high-mannose-type glycans for analyses of glucosidase II and UDP-Glc:glycoprotein glucosyltransferase

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The folding of glycoproteins is primarily mediated by a quality control system in the ER, in which glucosidase II (G-II) and UDP-Glc:glycoprotein glucosyltransferase (UGGT) serves as a key enzymes. In order to conduct precise analyses of these enzymes, we established systematic synthetic route to high-mannose-type glycans (ref. 1). Our analysis using synthetic substrates revealed glycan specificities of G-II (ref. 2) and UGGT (ref. 3). The inhibitory activities of various glycans suggest that UGGT has a strong affinity for the core pentasaccharide (Man_3-$GlcNAc_2$) of high-mannose-type glycans. Our comparison of the reactivity of acceptors that have been modified by various aglycons supports the hypothesis that UGGT recognizes the hydrophobic region of client glycoproteins. Moreover, we discovered fluorescently labeled substrates that will be valuable for highly sensitive detection of UGGT activity (ref. 4). In addition, analysis using various analogues of UDP-Glc revealed that UGGT has narrow donor specificity.

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Program/Abstract# 19

Side-chain conformations as mediators of saccharide structure and function: *N*-Acyl groups in aminosugars

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A distinguishing feature of biologically relevant saccharides is their high content of lone-pair electrons, contributed mainly by oxygen atoms within pyranosyl/furanosyl rings and as ring substituents (*e.g.*, exocyclic OH groups and *O*-glycosidic oxygens). The conformational properties of exo- and endocyclic C-O bonds allow different displays of lone pairs in free and bound forms, inducing structural changes that in turn influence reactivity. Recently, concerted use of multiple NMR *J*-couplings sensitive to exocyclic hydroxymethyl (-CH_2OH) conformation has been made to investigate correlated conformation within this substituent (Thibaudeau *et al.*, *J. Am. Chem. Soc.*, **2004**, *126*, 15668–15685). Combined experimental and theoretical studies have led to quantitative understandings of substituent effects on *J*-couplings involving exchangeable OH protons and on trans-glycoside $^3J_{COCC}$ values sensitive to glycosidic linkage conformation (for example, see: Zhao *et al.*, *J. Org. Chem.* **2007**, *72*, 7071–7082; Zhao *et al.*, *J. Org. Chem.* **2008**, *73*, 3255–3257). Described herein are the results of NMR investigations of the configurational and conformational behaviors of saccharide exocyclic *N*-acyl groups. *Cis-trans* isomerization (CTI) within the *N*-acyl sidechain of glucosamine anomers was studied using multiply ^{13}C-labeled *N*-formyl (NFm)- and *N*-acetyl (NAc)-D-glucosamines, yielding quantitative $K_{trans/cis}$ equilibrium constants in GlcNFm and GlcNAc anomers at different solution temperatures. ^{13}C NMR saturation transfer methods were used to measure CTI rate constants in

GlcNFm and GlcNAc anomers at different solution temperatures. In complementary studies, integrated experimental and theoretical (density functional theory) tools yielded new equations correlating multiple *J*-couplings involving ^{1}H, ^{13}C and ^{15}N with conformation about the C2-N bond of GlcNAc and related structures. These findings will be placed into the wider perspective of overall oligosaccharide conformation/dynamics in solution and their relationships to biological function.

Program/Abstract# 20

Involvement of N-acetylneuraminic acid in the regulation of GABA-uptake activity of GABA-transporter 1

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GABA (γ-aminobutyric acid) is the major inhibitory neurotransmitter in the central nervous system (CNS). GABA re-uptake by GABA transporters from the synaptic cleft is one important mechanism in the regulation of GABA activity, which is involved in a lot of diseases such as Parkinson's disease, epilepsy, chorea Huntingtone and schizophrenia. The GABA transporter 1 (GAT1) belongs to the family of Na+and Cl–coupled transport proteins, which possess 12 putative transmembrane domains and three N-glycosylation sites in the extracellular loop between the transmembrane domain 3 and 4. Previously we have determined that N-linked oligosaccharides side chains of GAT1, in particular their terminal structures, are involved in the process of GABA-transport of GAT1 by its affinity with sodium ions (Cai *et al*., 2005).

In this work, the role of the terminal N-acetylneuraminic acid of N-linked oligosaccharide of GAT1 in the GABA-reuptake is further investigated. GAT1 was stably expressed in CHO, CHOLec3 (a cell line with defection in N-acetylneuraminic acid biosynthesis) and Hek-293 cell lines. The expression of GAT1 protein was characterized. The GABA-uptake activity was determined in Hek-293/GAT1 with and without the treatment with neuraminidase. Treatment of the Hek-293/GAT1 cells with 1 unit of neuraminidase for three days, the N-acetylneuraminic acid of GAT1 could be detected hardly by lectin staining and the GABA-uptake was reduced to about 30%. On the other hand, the GABA-uptake of CHOLec3/GAT1 exhibited only 25% of that of CHO/GAT1. This suggests that deficiency of terminal N-acetylneuraminic acid of N-linked oligosaccharide results in reduction of GABA-uptake activity of GAT1 indicating an involvement of N-acetylneuraminic acid in the regulation of GABA-uptake of GAT1.

Program/Abstract# 21

Shigella O-specific oligosaccharide-core-protein conjugates: new vaccine candidates

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Shigellae, Gram-negative bacteria, cause endemic and epidemic diarrhea and/or dysentery worldwide, especially in developing countries, and especially in children. Control of this disease is hindered by the lack of safe drinking water and food, and the low infectious dose of this pathogen. Despite its discovery over a century ago, there is still no licensed vaccine against shigellosis. We hypothesized that serum IgG antibodies to the O-specific polysaccharide (O-SP) domains of the lipopolysaccharides (LPS) of these organisms would confer protection from infection and have shown that O-SP-protein conjugates were protective in young adults. To enhance immunogenicity of these conjugates we devised a new method of their preparation.

We present the isolation, structural characterization and conjugation of low molecular mass O-SP-core (O-SPC) fragments of *S. sonnei*, *S. dysenteriae* type 1 and *S. flexnerii* type 2a and 6. The structure of each O-SPC fragment, containing between 1 to 5 O-SP repeat units plus core, was determined by NMR and mass spectroscopy. Next, O-SPC fragments of different length from each strain were bound to the protein carrier by their reducing ends. The conjugation was based on the formation of oxime linkages between the terminal Kdo residues of the shigellae saccharides and aminooxy linkers bound to the protein, and carried out at a neutral pH, room temperature and in a short time. All conjugates, injected as saline solutions at a fraction of an estimated human dose, induced IgG against homologous LPS in young outbred mice. Accordingly, we propose to evaluate clinically these new conjugates.

Program/Abstract# 22

Assay for the activity of C5-epimerase using engineered 2-O-sulfotransferase

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Heparan sulfate (HS) is involved in essential physiological functions such as nutritional metabolism, cell growth, wound repair and blood coagulations, as well as patho-physiological functions such as cancer and viral infections. HS is a highly sulfated polysaccharide consisting of glucuronic acid (or iduronic acid) linked to glucosamine carrying various sulfo groups. The functional diversity of HS results from the different sulfation patterns and the distribution of iduronic acid. The biosynthetic pathway of HS involves a series of specialized sulfotransferases and an epimerase. The HS C_5-epimerase catalyzes the conversion from glucuronic acid to iduronic acid, which is a critical monosaccharide unit for the functions of HS. However, the method for determining the activity has been tedious and insensitive. Here, we report a two-enzyme coupling assay to determine the activity of C_5-epimerase using an engineered 2-*O*-sulfotransferase. While the wild type 2-O-sulfotransferase sulfates both glucuronic acid and iduronic acid units, the single point mutant of 2-*O*-sulfotransferase (2OSTY94I) transfers sulfate to the iduronic acid only. *N*-sulfated heparosan is a polysaccharide containing no iduronic acid. Incubating N-sulfated heparosan with C_5-epimerase converts some of the glucuronic acid to iduronic acid, thus it becomes a substrate for 2OSTY94I. Determining the susceptibilities of 2OSTY94I to the C_5-epimerase-treated N-sulfated heparosan directly correlates to the activity of C_5-epimerase. The new method can be used to assay the activities of various C_5-epimerase mutants with high throughputs. The method will significantly reduce the complexity for assaying the activity of C_5-epimerase and facilitate the structural and functional analysis of C_5-epimerase.

Program/Abstract# 23

Importance of differential N-glycosylation on a5ß1 integrin

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Integrins are cell surface transmembrane glycoproteins that function as adhesion receptors in cell-ECM interactions and link matrix proteins to the cytoskeleton. Although integrin-mediated adhesion is based on the binding of α and β subunits to a defined peptide sequence, the strength of this binding is modulated by various factors including the status of glycosylation of integrin. For example, integrins undergo glycosylation by N-acetylglucosaminyltransferase III (GnT-III), resulting in the inhibition of cell migration. Whereas integrins glycosylated by GnT-V to form β1,6 GlcNAc branch, contribute to the promotion of cell migration. Recently, we found that the N-glycosylation on β-propeller domain of the α5 subunit, in particular sites number 3–5 sites (α5S3-5), is essential for its hetero-dimer formation and its biological functions such as cell spreading and cell migration, as well as the proper folding of α5 subunit. Interestingly, the site-4 of the α5 is essential and effective for GnT-III modification among 14 potential N-glycosylation sites. On the other hand, we also found that N-glycosylation of the I-like domain (S4-6) of the β1 subunit is essential to α5β1 heterodimer formation and biological function of the subunit. Moreover, because the α5S3–5/β1S4–6 mutant represents the minimal N-glycosylation required for functional expression, it might also be useful for the study of molecular structures.

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Program/Abstract# 24

Total synthesis without protecting groups: imino sugars as glycosidase inhibitors

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Owing to their properties as inhibitors of carbohydrate processing enzymes, imino sugars have enormous therapeutic potential in diseases such as viral infection, bacterial infection, lysosomal storage disorders and diabetes. Herein we report a protecting group free synthesis of 2,3-*cis* substituted hydroxypyrollidines [1], and recent syntheses of other biologically important imino sugars. In the course of this work two novel reaction methodologies were developed: one for the stereoselective formation of cyclic carbamates from olefinic amines and the other for the formation of primary amines without the need for protecting groups.

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Program/Abstract# 25

Hyaluronan in tissue remodeling

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Hyaluronan (HA) is a large polysaccharide which resides on cell surfaces and in the extracellular matrices. HA facilitates diffusion of small molecules and cell migration and proliferation. In skin, wounding causes a rapid transient upregulation of HA-synthases and accumulation of HA both in epidermis and dermis, while later during the healing phase HA is removed. Similarly chronic UVR causes accumulation of HA in the skin. Inflammation causes the pericellular HA-coat to acquire an extended conformation, which regulates the activity of inflammatory cells. Inhibition of HA-synthesis reduces HA-dependent monocyte adhesion to cytokine treated keratinocytes, and inflammatory cell infiltration to skin granuloma tissue *in vivo*. HA accumulation is most conspicuous in malignancies that develop in tissues normally devoid of HA, such as single layered epithelia and their HA-poor stroma. Tumor progression is likely when HA is abundant in the tumor cells of gastric and colon carcinomas. Likewise, HA accumulation in the peritumoral stroma of breast and ovarian carcinomas indicates a bleak prognosis for the patient. In cancers originating from stratified epithelia there is a dual phase response in cancer-cell associated HA: in premalignant lesions the HA-content is increased while in poorly differentiated SCC's it decreases. It is thus obvious that HA-accumulation is an inherent feature in most epithelial malignant tumors possibly contributing to tumor growth by rendering the tumor cells more aggressive. The inhibition of HA-synthesis by 4-MU retards the progression of experimental melanoma, and renders Trastuzumab resistant tumors responsive to the drug. Therefore, in the future we may see hyaluronan inhibitors as one asset in the battle against cancer.

Program/Abstract# 26

D-Arabinosyltransferases are druggable targets in tuberculosis: lessons learnt from Ethambutol

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Nearly a third of the human population is infected asymptomatically with *Mycobacterium tuberculosis*(*M.tb*), one of the most successful pathogens of mankind. Tuberculosis (TB) can now be effectively treated by modern chemotherapy, Yet there has been an increase in drug resistant TB. Therefore, search for new targets and new drugs cannot be understated. Despite recent successes in the discovery of new anti-TB drugs such as diaryl quinoline, TMC207 and promise of nitroimidazole and nitrofuran compounds, more efforts are required to find better drugs to be used in combination with existing therapy to reduce treatment duration and avert resistance. The premise is to discover antibacterials that target multiple enzymes of a single metabolic pathway that cause sequential blockade in the synthesis of an essential bacterial component. Used in combination, these antibacterials may delay the emergence of resistance, a strategy that might prove viable to extend the clinical utility of new anti-TB agents. The majority of the glycosyltransferases involved in the synthesis of the common and rare sugars of the cell envelope of *Mycobacterium* spp. are essential for viability and conceivably are suitable targets for new drug development against multiple and extensive drug resistant tuberculosis (MDR- and XDR-TB). Ethambutol (EMB), a first-line antituberculosis drug, targets cell wall **D**-arabinan synthesis, the major polysaccharide of mycobacterial cell wall, by interfering with the polymerization and induces the accumulation of β-D-arabinofuranosyl-P-decaprenol (DPA)-an intermediate in arabinan synthesis. With chemical biology and genomic tools, four to five new arabinosyltransferases (AraT) are now revealded aside from the Emb proteins. Two specific essential AraTs-AftC and AftB, which are involved in internal branching and terminal β-capping of the D-arabinan and are <u>insensitive</u> to EMB and are validated targets for therapeutic intervention.

Program/Abstract# 27

ß3GlcNAc-T5 induction in gastric epithelial cells by helicobacter pylori leads to expression of sialyl-Lewis X, the ligand for SabA adhesin

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A long-term infection by *Helicobacter pylori* (*Hp*) is associated with the development of gastric carcinoma. *Hp* can produce phenotypic alterations in gastric epithelial cells. Expression of the inflammation-associated sialyl-Le^x antigen in the gastric epithelium has been shown to be induced during persistent *Hp* infection, suggesting that *Hp* may trigger the host tissue to retailor the gastric mucosal glycosylation patterns to a more favorable environment for its adhesion. *Hp* has been shown to adhere to glycoconjugates expressed in the gastric mucosa through bacterial adhesins (BabA, SabA). This study evaluated the epithelial gene expression in response to *Hp* infection. Our results showed that *Hp* induced significant alterations in 168 of the 1031 genes tested in a microarray platform. The most virulent *Hp* strain led to altered expression of glycosylation-related genes, including the increased expression of β3GlcNAc-T5, a glycosyltransferase

involved in the synthesis of Lewis determinants. Further evaluation of a panel of different *Hp* strains showed that β3GlcNAc-T5 overexpression was elicited only by the virulent *Hp* strains (cagPAI+) [1]. β3GlcNAc-T5 overexpression in transfected gastric cell lines leads to increased expression of sialyl-Le^x antigen and increased the adhesion of *Hp*[1]. In conclusion, our results show that highly pathogenic *Hp* strains induce β3GlcNAc-T5 and that the expression of this enzyme leads to sialyl-Le^x expression suggesting a mechanism by which *Hp* modulates the synthesis of the SabA adhesin ligand, essential to achieve long-term successful colonization.

Supported by FCT (POCI/SAU-OBS/56686/2004 and PDTC/CTM65330/2006). Resources provided by the CFG were funded by NIGMS-GM62116.

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Program/Abstract# 28

Glycan biosynthesis in the pathogenic fungus *Cryptococcus neoformans*

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Cryptococcus neoformans is a serious opportunistic pathogen. The main virulence factor of this fungus is a prominent polysaccharide capsule that is required for virulence and associates with the cell wall via alpha-glucan. The two capsule polysaccharides are GXM (glucuronoxylomannan) and GXMGal (glucuronoxylomannogalactan), named after their components. One major question of capsule biosynthesis is where it is made. Our studies indicate that GXM components are generated within the cell and travel to the surface via the secretory pathway. We also find that changes in capsule size are mediated at the polymer level, before secretion, rather than by alterations in capsule assembly. Because cells lacking UDP-xylose produce stunted capsule fibers and are avirulent in animal models, we are investigating cryptococcal xylosyltransferases. We have purified a beta-1,2-xylosyltransferase with specificity appropriate for incorporation of xylose into capsule polysaccharides. Intriguingly, the corresponding gene has multiple homologs in fungi, but none in other kingdoms. Gene deletion yields cells that produce GXM with reduced beta-1,2-xylose content and GalXM which is almost devoid of this linkage. Xylose is also completely absent from cryptococcal glycoinositolphosphoceramides, indicating the enzyme functions in synthesis of three distinct glycoconjugates. Significantly, the mutant shows reduced growth in a short-term mouse model of infection. *C. neoformans* expresses a second xylosyltransferase with similar in vitro activity but with a less prominent role in capsule synthesis. A third transferase has the novel activity of adding xylose-phosphate to mannose residues, most likely as part of cryptococcal O-glycan synthesis. These processes offer a glimpse of the fascinating glycobiology of *Cryptococcus neoformans.*

Program/Abstract# 29

Characterisation of trans-sialidase genes from Trypanosoma congolense

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Like other African trypanosomes, *T. congolense* express trans-sia-li-da-ses (TS) in the procyclic form in the insect vector (1), where the sialylation appears to improve the survival rate dramatically (2).

Based on a partial sequence (3) and an open reading frame (TS1) in the WTSI data base, we amplified DNA sequences encoding for seven proteins with overall 83% amino acid identity. Several of the resulting amino acid changes were found in the predicted active site of the enzyme. Further database searches confirmed the presence of these TS1 gene copies in the *T. congolense* genome and provided evidence for at least 5 additional TS-like genes having only 50–60% amino acid identity with TS1. All these TS-like sequences were clearly more closely related to each other than to the T. brucei TS genes (4).

Four of the putative TS were expressed as recombinant proteins, isolated and assayed for trans-sialylation activity. Three of the purified proteins desialylated fetuin only in the presence of lactose and pro-duced 2,3-sialyllactose, clearly demonstrating their TS activity.

In summary, our observations demonstrated that the heterogeneity of TS in *T. congolense* is some-what more complex than in *T. brucei*. Of particular interest are the 7 almost identical copies of one gene with different enzymatic activities raising the question of what their functions might be.

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2) Nagamune *et al*. (2004), J. Exp. Med. 199:1445–1450
3) Tiralongo *et al*. (2003), Biol. Chem. 384: 1203–1213
4) Montagna *et al*. (2002), Eur. J. Biochem. 269: 2941–2950

Program/Abstract# 30

Initiation and polymerization of bacterial polysialic acids on hydrophobic acceptors

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Bacterial polysialic acids are virulence factors for pathogenic bacteria that cause meningitidis and upper urinary tract infections. *Neisseria meningitidis* group B and C are the major causes of meningococcal disease in industrialized countries. Vaccines against *N. meningitidis* group C are based on its α2,9 linked polysialic acid capsular polysaccharide and are effective at preventing disease. The α2,8 linked polysialic acid of *N. meningitidis* group B is a self antigen and therefore not an effective vaccine. The polysialyltransferases of these bacteria are firmly associated with the cytoplasmic membrane and do not initiate polysialic acid synthesis de novo. We have shown that these polysialyltransferases initiate polysialic acid synthesis on a hydrophobic acceptor. This acceptor is present in the membranes of acapsular mutants *E. coli* K1 and *N. meningitidis* group B mutants. We show that an acapsular mutant with a defect in the *N. meningitidis* group B polysialyltransferase, produces outer membrane vesicles containing an acceptor for the α2,9 polysialyltransferase. This acceptor is an amphipathic molecule and can be elongated to produce polysialic acid reactive with group C specific antibody. We have compared the polymerization reaction of polysialyltransferases of *E. coli* K1 and K92 and the *N. meningitidis* Group B and C using fluorescence based HPLC assay. All of these polysialyltransferases will extend an oligosaccharide containing α(2,8) linked disialic acid and a hydrophobic aglycon. The polysialyltransferases extend these substrates to form long polymers in a non-processive fashion. The polysialyltransferases will also extend oligosialic acids but have a preference for oligosialic acids with a hydrophobic end group. This result is consistent with the *in vivo* acceptor having a hydrophobic component.

Program/Abstract# 31

Analysis of trypanosoma cruzi GPI10 - a multifunctional GPI mannosyltransferase

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The surface of *Trypanosoma cruzi*, the species of euglenoid trypanosomes that causes Chagas disease, is covered with glycoconjugates, the majority (including the variant surface glycoprotein) being attached to the parasite via a glycophosphotidylinositol (GPI) anchor. These help protect the organism from host immune responses, and are potent pro-inflammatory compounds. Analysis of *T. cruzi* GPI anchor structure suggests that the glycan consists of four mannoses attached to glucosamine; which is similar to the structure of Saccharomyces cerevisiae GPIs. Searches of the *T. cruzi* genome for genes encoding proteins homologous to known GPI mannosyltransferases has led to the identification of proteins likely to add the first (Tc*GPI14*), second (Tc*GPI18*) and third (Tc*GPI10*) mannoses to the GPI glycan. However, no sequence homolog of a fourth mannosyltransferase (eg. *SMP3/PIGZ*) is present. Using a yeast complementation approach, we set out to identify the gene product responsible for fourth mannose addition in *T. cruzi*. We found that the putative third mannosyltransferase, Tc*GPI10*, could complement lethal deletions of both the *S. cerevisiae* third mannosyltransferase (Sc*gpi10*Δ), and fourth mannosyltransferase (Sc*smp3*Δ), suggesting that TcGpi10 is capable of adding both α1,2-linked mannoses to the GPI glycan. This is the first example of a multifunctional GPI anchor biosynthesis protein. Future studies of TcGpi10 will provide a better understanding of both *T. cruzi* GPI anchor biosynthesis, as well as the enzymology of Dol-P-Man utilizing α1,2 mannosyltransferases.

Program/Abstract# 32

Lipopolysaccharide assembly in marine bacteria: definition of ensemble lipid heterogeneity by Vibrational cooling fourier transform mass spectrometry

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Intrinsic heterogeneity of the lipid terminal or lipid A (LA) in the lipopolysaccharide (LPS) of pathogenic and environmental bacteria causes a paint brush-like profile in their mass spectra. This phenomenon provides clear evidence that the LPS molecules carry a wide variety of LAs. The molecular design of the lipid terminal in the intact LPS of a marine bacterium *Pseudoalteromonas haloplanktis* ATCC 14393^{T} has been deciphered using several MS techniques: electrospray (ESI) and vibrationally cooled matrix-assisted laser desorption/ionization (VC-MALDI) together with infrared multiphoton dissociation (IRMPD) and sustained off-resonance irradiation collisionally activated decomposition (SORI-CAD). The LPS lipid isolated showed an “intrinsic” heterogeneity in each of 6 ion clusters that were found to differ in the numbers of phosphate and acyl groups and in the presence of 4 types of hydroxyl fatty acyl substituents. The enigmatic profile for the lipid appears to result from an enzyme convolution involving a pool of ACP-acyl precursors. Lipid molecules of the ensemble differed widely in the total primary acyl chain length and this presented an obstacle to detect the full distribution by both MS ionization techniques without distortion of the ensemble pattern. Partial de-*O*-acylation and de-phosphorylation during isolation of the lipid terminal also masked the native image. VC-MALDI-FTMS/

MS using a TLC plate as the target proved to be the indispensable method for recognizing and fully defining 3 species of the lipid ensembles that are composed of at least 27 molecular types. (Supported by NIH-NCRR P41 RR10888)

Program/Abstract# 33

Stable-isotope-assisted NMR approaches to structural glycomics

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Recent advances in structural glycomics have enabled us to collect information on glycoforms, *i.e.* sequences, linkage, positions and profiling of glycans, of glycoproteins in systematic manners. One of the central issues in the next stage of structural glycomics is to provide structural basis of the biological functions of the individual glycoforms of glycoproteins. NMR spectroscopy has great potential to provide us with information on structures, dynamics, and interactions at atomic resolution of glycoconjugates. Recently, ultra-high field NMR spectrometers (beyond 900 MHz) have become available for applications to structural glycobiology. Utility of ultra-high field NMR is undoubtedly expanded by use of stable isotope labeling of the sugar chains.

In this presentation, we will illustrate several examples of application of the stable-isotope-assisted NMR approach to structural analyses of glycoprotein glycans. We have also successfully applied ultra-high field NMR spectroscopy in conjunction with stable isotope labeling to characterization of the conformation of amyloid β bound to GM1 clusters, which is relevant to pathogenic mechanism of Altzheimer's disease. This line of investigation can be extended to detailed structural analyses of carbohydrate-protein and carbohydrate-carbohydrate interactions.

This work was supported by CREST/JST.

Program/Abstract# 34

New approach for Glyco-and lipidomics - imaging technology of molecular species of brain gangliosides by combination of TLC-Blot and MALDI-TOF MS

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We have developed TLC-Blot system which made possible direct analysis of blotted glycolipids on PVDF membrane from HPTLC-plate by immuno-staining, chemical staining, enzyme treatment and mass spectrometric procedure. Recently, HPLC/MS system has been widely used for lipidomics, however the conditions for the separation of individual components are complex, and difficult to control. TLC is a classical method but still the best technology for clear separation of each component. We have found a good solvent system for the transfer of gangliosides from HPTLC plate to PVDF membrane and these separated components are visible by treating the membrane with primuline reagent. We scanned blotted bands on PVDF membrane with QIT MALDI-TOF MS equipment and expressed as molecular imaging. The molecular species of human brain gangliosides are clearly distributed in individual ganglioside bands. Hydrophobic properties are quite important for membrane fluidity and molecular assembly to maintain the cell behavior including cell growth, differentiation and cell-cell interaction. Gangliosides in nervous system have been reported to be involved in the learning and memory. In the present ,study we show a new approach for Glyco- and Lipidomics with imaging technology.

Program/Abstract# 35

Latest advancements in HPLC-Chip/MS with applications to glycomics research

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Recently a new fully automated and integrated analytical system consisting of a chip-based chromatography system in conjunction with ion trap, time-of-flight, triple quadrupole and quadrupole time-of-flight mass spectrometers was introduced. The microfluidic HPLC-chips are made of laser ablated and laminated biocompatible polyimide films. Sample enrichment, separation and nanoelectrospray tips are fully integrated in the chip device. Chips with different functionality can be easily designed and developed for specific LC/MS applications. The system represents a breakthrough in nanoelectrospray MS sensitivity, chromatographic separation, reproducibility, sample throughput and ease of use. In this presentation an update about the latest technical developments of the chip-MS system will be provided. Applications of the Chip-MS system to the study of free, N-linked and O-linked glycans including MRM and negative ion mode measurements will be presented. Finally, a new chip will be introduced that automates the entire workflow for the analysis of glycans from N-glycoproteins. On-chip digestion is performed within 10 s and the entire analysis time from sample injection into the system to mass spectrometry readout is only 10 min.

Program/Abstract# 36

A novel strategy for glycomic characterization of mucins using supported molecular matrix electrophoresis

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Mucins are the major constituents of epithelial mucus and are typically characterized by large molecular mass (~ 2 MDa) and high carbohydrate content (50~90% by weight) reflecting heavy glycosylation with a large number of *O*-linked glycans. Cancer-associated alterations of glycans are often found on mucins. Mucins and their glycans are therefore considered to be a useful diagnostic marker for the early detection of various cancers and a means of specific discrimination between cancers and benign diseases. However, mucins have been left behind by modern techniq ues in terms of characterization due to their large size, polymeric nature, and heterogeneous glycosylation. Here we introduce a novel strategy for the separation and characterization of mucins using a new technique, named "supported molecular matrix electrophoresis (SMME)" (1). SMME is a new method of electrophoresis in which analytes migrate in a molecular matrix which is supported by backbone materials. The matrix molecules and the backbone materials can be arranged depending on the nature of the analyte and the purpose of the experiment. For separation and characterization of mucins, we used a PVDF membrane and poly(vinyl alcohol) (PVA) as the backbone material and the matrix molecule, respectively. For electrophoresis, an apparatus for conventional cellulose acetate membrane electrophoresis was used. Combining SMME with mass spectrometry, we analyzed mucins in clinical pancreatic juice samples which are important source for biomarker of pancreatic disorders including tumors. We show some results from the glycomic characterization of these mucins to demonstrate the feasibility of the strategy for biomarker exploration based on mucin-lycomics.

(1) Matsuno, YK. *et al*. (2009) *Anal. Chem.* 81, 3816–3823.

Program/Abstract# 37

Enrichment and glycoproteomic characterization of N- and O-glycopeptides from cerebrospinal fluid

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The analysis of glycoproteins with respect to glycan structures, glycan attachment sites and protein identities is desirable in the Glycobiology field. Glycoproteomic analysis of biological samples is however challenging due to the complexity of tryptic digests, low signal intensity and structural microheterogeneity associated with glycopeptides. We present a novel strategy for the purification of glycopeptides from biological fluids, and the use of mass spectrometry to analyze the glycopeptides. Glycoproteins were periodate oxidized on sialic acids and captured to hydrazide beads. The glycoproteins were trypsin digested and the glycopeptides were released by acid hydrolysis, exploiting the acid sensitive glycosidic bond of sialic acids. The released asialoglycopeptides were analyzed by nanoLC-LTQ-FTICR with a mass accuracy in the low ppm range and were subjected to multiple rounds of CID-fragmentation from which glycan- and peptide sequences were elucidated.

This capture-and-release method was applied on cerebrospinal fluid (CSF) samples from three healthy individuals. 34 unique N-glycosylation sites from 24 glycoproteins were characterized along with 42 O-glycosylation sites from 21 glycoproteins. The majority of O-glycopeptides have not been reported previously and findings include O-glycosylation of Cystatin C, Dystroglycan and Apolipoprotein E. All found O-glycans from CSF were composed of HexHexNAc in support of the original presence of sialylated core 1 structure, Neu5Acα3Galβ3GalNAc-*O*-Ser/Thr. For N-glycans the complex biantennary structure was the most common. In conclusion, we describe a simple approach for the fast and selective purification of glycopeptides from complex biological samples for mass spectrometric analysis.

Program/Abstract# 38

Current challenges in glycosylation pattern analysis of snails

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Currently, one of the most exciting fields of biochemistry is glycomics. Due to the large heterogeneity of glycans within this taxonomic rank, the class of gastropods is especially fascinating. As subject of research we use a wide variety of water and land snails such as *Achatina fulica, Arion lusitanicus, Arianta arbustorum, Biomphalaria glabrata, Cepaea hortensis, Limax maximus, Lymnea stagnalis* or *Planorbarius corneus*.

Glycosylation pattern analysis of whole proteins was carried out by Western blotting using various lectins with well determined binding specificities. Sialylation potential was investigated by *Sambucus nigra* agglutinin and

Maackia amurensis agglutinin, fucosylation potential was investigated by *Aleuria aurantia* lectin, *Lens culinaris* agglutinin, *Lotus tetragonolobus* agglutinin and *Ulex europaeus* agglutinin.

The release of glycans was carried out enzymatically with glycopeptide N-glycosidase (PNGase A or PNGase F) in the case of N-glycans and chemically using β-elimination in the case of O-glycans. The glycans were analyzed by two-dimensional HPLC after labeling with 2-aminopyridine or by MALDI-TOF-MS/MS and ESI-IT-MS^n after permethylation. Monosaccharide composition analysis was carried out after glycan hydrolysis and fluorescent tagging with anthranilic acid or 3-methyl-1-phenyl-2-pyrazolin-5-one.

Combining the results, snails seem to be capable to produce a broad spectrum of structures ranging from those typical in mammals through to structures similar to those found in plants, insects or nematodes. The detailed knowledge of the very complex glycosylation system of gastropods will be a valuable tool to understand the principle rules of glycosylation in all organisms.

This project is supported by the FWF project P20393-B11.

Program/Abstract# 39

Glycosaminoglycans in vertebrate development: form, function and future

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Glycosaminoglycans (GAGs) form a significant proportion of the extracellular matrix (ECM), and have been implicated in every aspect of embryogenesis, from the formation of the cumulus-oocyte-complex (COC) to the regulation of developmental processes in organs as diverse in form and function as the brain, heart, kidney and limb. GAGs are key regulators of vertebrate development, demonstrating diverse mechanisms that drive these effects. Thus, high molecular weight forms have structural roles in tissue hydration and physical regulation of molecules at the cell surface that alter gene expression. Further, the interaction of lower molecular weight GAGs with specific receptors alters cell behavior such as migration and proliferation. In certain situations, GAGs regulate epithelial- and endothelial-mesenchymal transition important for appropriate organogenesis. GAGs also modulate intracellular signaling pathways leading to the regulation of transcription factors, gene expression, and cell division. Importantly, GAGs regulate key developmental processes such as angiogenesis. The discovery of enzymes regulating GAG content, synthesis and molecular size has opened an increasing complexity to the mechanisms regulating development. Key information gleaned from transgenic and knockout mice is unraveling important contributions by these systems to vertebrate development. These processes and specific examples will be highlighted and discussed.

Program/Abstract# 40

Regulation and pathophysiological implications of UDP-GlcNAc 2-epimerase/ManNAc kinase (GNE) as the key enzyme of sialic acid biosynthesis

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The key enzyme for the biosynthesis of N-acetylneuraminic acid, from which all other sialic acids are formed, is the bifunctional enzyme UDP-N-acetylglucosamine 2-epimerase/N-acetylmannosamine kinase (GNE). GNE is a highly conserved protein found throughout the animal kingdom. Its highest expression is seen in the liver and placenta. GNE is regulated by a variety of biochemical means, including tetramerisation promoted by the substrate UDP-GlcNAc, phosphorylation by protein kinase C and feedback inhibition by CMP-Neu5Ac, which is defect in the human disease sialuria. GNE knock-out in mice leads to embryonic lethality, emphasizing the crucial role of this key enzyme for sialic acid biosynthesis. The metabolic capacity to synthesize sialic acid and CMP-sialic acid upon ManNAc loads is amazingly high. An additional characteristic of GNE is its interaction with proteins involved in the regulation of development, which might play a crucial role in the hereditary inclusion body myopathy. Due to the importance of increased concentrations of tumor-surface sialic acid, first attempts to find inhibitors of GNE have been successful.

Program/Abstract# 41

***O*-GlcNAc protein modification in cancer cells increases in response to glucose deprivation through glycogen degradation**

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In general, the level of UDP-GlcNAc and of protein *O*-GlcNAc modification (*O*GlcNAcylation) decreases following glucose deprivation. However, recent reports demonstrated increased *O*-GlcNAcylation by glucose deprivation in HepG2 and Neuro-2a cells. Here, we report increased *O*GlcNAcylation in non-small cell lung carcinoma A549 cells and various other cells in response to glucose deprivation. Although the level of *O*-GlcNAc transferase (OGT) was unchanged, the enzyme contained less *O*GlcNAc and its activity was increased. Moreover, *O*-GlcANcase (OGA) activity was reduced. The studied cells contain glycogen and we show that its degradation in response to glucose deprivation provides a source for UDP-GlcNAc required for increased *O*GlcNAcylation under this condition. This required active glycogen phosphorylase and involved increased glutamine:fructose-6- phosphate amidotrasnferase activity, the first and rate-limiting enzyme in the hexosamine biosynthetic pathway. Interestingly, glucose deprivation reduced the amount of phosphofructokinase 1, a regulatory glycolytic enzyme, and blocked ATP synthesis. These findings suggest that glycogen is the source for increased *O*-GlcNAcylation but not for generating ATP in response to glucose deprivation and that this may be a mechanism for cancer cell survival.

Program/Abstract# 42

Age-associated translocation of glycoprotein cathepsin D caused by oxidative stress and proteasome inhibition

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We reported previously that *N*-linked glycoproteins were accumulated in the cytosol of the normal aging rat brain, and that one protein had been identified as cathepsin D (Mech. Ageing Dev, 127, 771–778, 2006). In this study, to elucidate the mechanism of cathepsin D accumulation in the cytosol, we examined the effects of oxidative stress and proteasome inhibition on the apoptosis and subcellular localization of cathepsin D in primary cultured neurons and astrocytes. We found that oxidative stress and proteasome inhibition induced apoptosis and demonstrated that cathepsin D was translocated from lysosomes to cytosol in both cells. These results suggested that oxidative stress and the suppression of proteasome activity triggered the translocation of cathepsin D from lysosomes to cytosol that they are responsible for the age-related accumulation of cathepsin D in the cytosol. Although it is unknown whether cathepsin D accumulated in the cytosol mediates neural or astrocytic cell death directly, cytosolic cathepsin D would be a biomarker of age-related damage in the brain.

Program/Abstract# 43

A new function of polysialic acid on NCAM as the reservoir for the particular groups of neurotrophins, growth factors, and neurotransmitters that regulate the neural activity

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Polysialic acid (polySia) is a unique polymer of sialic acid. PolySia is expressed in embryonic brains during neural differentiation and mostly disappears in adult brain, although the neural cell adhesion molecule (NCAM) expression level remains unchanged. PolySia on NCAM has anti-adhesive effects on the cell-cell/extracellular matrix interaction due to its bulky polyanionic nature and is involved in neural cell migration, axonal guidance, fasciculation, myelination, synapse formation, and functional plasticity of the nervous system. Interestingly, polySia-NCAM remains in adult brains, especially in distinct regions such as hippocampus, hypothalamic nuclei, and olfactory system where neurogenesis is ongoing. However, functional importance of polySia-NCAM in adult brain remains largely unknown. As a new function of polySia in adult brain, we hypothesize the reservoir function of polySia toward various molecules that are deeply involved in the neural activities in adult brain. In this study, we obtained three lines of evidence demonstrating our hypothesis. First, we demonstrated that a brain-derived neurotrophic factor (BDNF) dimer binds directly to polySia to form a large complex under physiologic conditions and that the BDNF, after making complex with polySia, can bind to the BDNF receptors, TrkB and p75NTR (1). Second, we showed that polySia also interacts with FGF-2 in a different manner than BDNF. Third, we further demonstrated that polySia binds with a particular group of neurotransmitters that might be co-localized with polySia-NCAM in some synaptic cleft by frontal affinity chromatography.

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Program/Abstract# 44

Insights into neural cell metabolism from NMR

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Limited knowledge is available about the mutual regulation of metabolic pathways in neurons on a molecular level. Prior

studies have focused on monitoring the fate of ^{13}C labelled metabolites occurring during the citric acid cycle or glycolysis. Metabolic products from the hexosamine pathway have attracted little attention so far. Yet, these intermediates are critical for the proper functioning of neurons. One such intermediate is UDP-GlcNAc which serves as a donor substrate for *O*-GlcNAc transferase that mediates the *O*-GlcNAcylation of cytosolic proteins [1]. At the same time UDP-GlcNAc can be further converted to CMP-Neu5Ac, the donor substrate for sialyltransferases. We are currently exploring the potential of UDP-GlcNAc and other metabolites of the hexosamine pathway as metabolic markers for the functional or pathological state of neural cells within the framework of the so called "Selfish Brain" theory that provides a new model of energy regulation within the human body. It states that the brain satisfies its own energy requirements first [2]. On a cellular basis energy production of neurons is described by the astrocyte-neuron lactate shuttle hypothesis [3]. Here, NMR spectroscopy in combination with selective isotope labelling of metabolic substrates is used to map the functional and the energy state of neurons, *i.e.* we follow the fate of metabolic species in cell extracts and supernatants [4,5]. As a model system we are using a neuronal cell line (HT-22).

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Program/Abstract# 45

Protein N-glycosylation in yeast: new insights with surprises

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The pathway of N-glycosylation occurs in an evolutionary conserved manner with the assembly of the lipid-linked oligosaccharide (LLO) precursor $Glc_3Man_9GlcNAc_2$-PP-Dol in the ER, the glycan moiety of which is subsequently transferred by the oligosaccharyltransferase complex (OST) onto selected Asn-X-Ser/Thr acceptor sites of the nascent polypeptide chain. The presentation will summarize identification and characterization of hitherto "missing" glycosyltransferases of the LLO pathway as well as new findings to OST. We found that the second step, forming $GlcNAc_2$-PP-Dol, is catalyzed by a dimeric complex in which membrane-bound Alg14 recruits cytosolic Alg13 to the cytosolic face of the ER. It can be replaced by a single polypeptide from *Leishmania* containing both domains. Investigation of Alg2 disclosed it as a bifunctional enzyme required for both the transfer of the α1,3- and α1,6-mannose residue to $Man_1GlcNAc_2$-PP-Dol to form $Man_3GlcNAc_2$-PP-Dol. Study of Alg2 topology predicts four transmembrane spanning helices. We prove that only the two N-terminal domains fulfill this criterion, whereas the C-terminal hydrophobic sequences contribute to ER localization in a non transmembrane manner. Surprisingly, none of the four domains is essential for activity, as truncated variants can exert their function as long as Alg2 is associated with the ER by either its N- or C-terminal hydrophobic regions. Alg11 has been identified as an enzyme with a dual function catalyzing the transfer of both α1,2-linked mannoses on the cytosolic side to form $Man_5GlcNAc_2$-PP-Dol before it flips to the luminal side of the ER. The pivotal step of protein *N*-glycosylation is catalyzed by the multimeric OST, which in yeast consists of nine subunits. We found that Stt3 from *Leishmania* can substitute for the whole complex as a free, monomeric enzyme with a broad specificity for non-glucosylated mannose-oligosaccharides, typical for protists. But when incorporated into the OST complex, *L.m*STT3 transfers also the common $Glc_3Man_9GlcNAc_2$-PP-Dol donor indicating that the complex determines the specificity.

Program/Abstract# 46

Ligand undetectable membrane glycosphingolipids: cholesterol can make GSLs "invisible"

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We developed a method for the generation of detergent resistant GSL-containing vesicles via mixing purified GSL with cholesterol in the presence of detergent and separation of the generated vesicles in a discontinuous sucrose gradient, containing an appropriate ligand, when unbound ligand remains at the original. Using this system, we were able to separate the ligand binding vesicles from the bulk of the GSL/cholesterol containing vesicles. The ligands studied included verotoxin 1 and 2, which bind the glycolipid, globotriaosyl ceramide, Mab anti-Gb_3, HIV gp120, which binds Gb_3, sulfatide and galactosyl ceramide, cholera toxin, which binds GM1 ganglioside and anti-GM1 antibodies. In all cases, the ligand binding GSL vesicles could be separated at the top of the gradient from the bulk of the GSL/cholesterol vesicles which ran at the 30–5% sucrose interface. Post-embedding immunoelectron microscopy confirmed ligand binding to the surface of vesicles at the gradient top but vesicles at the sucrose gradient interface only bound ligand within the core of

these larger vesicles These studies indicate that the majority of GSL present in the outer bilayer of these vesicles is unavailable for ligand binding. We propose that excess cholesterol can mask such membrane GSLs by altering the conformation of the carbohydrate moiety. This effect was mirrored in vesicles generated from the lipid extract of cultured cell membranes, but not in DRM extracted directly from cells, although in such cell DRM preparations, ligand binding in the gradient fractions was disproportional to their GSL content. For VT1, Gb_3 within the masked Gb_3/cholesterol vesicles could be made available for binding by inclusion of ceramide monohexosides. In separate studies, we found a carbohydrate/carbohydrate interaction between Gb_3 and such monohexosyl ceramides, which might provide a basis for the unmasking of the cholesterol obscured Gb_3 vesicles. Using a phospholipid GSL/liposome binding assay, we again found that incorporation of cholesterol resulted in GSL masking which was reversed in the presence of ceramide monohexoside. These studies indicate that cholesterol can mediate aglycone modulation of GSL receptor function but that GSL/GSL complexes may oppose this interaction to promote ligand binding.

Program/Abstract# 47

Glycan-regulated proteolysis in the secretory pathway

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The unfolded protein response boosts the efficiency of endoplasmic reticulum (ER)-associated degradation (ERAD) by impairing the proteolytic down-regulation of ER mannosidase I (ERManI) which targets misfolded glycoproteins for proteasomal degradation. Homozygous expression of the misfolded Z variant of alpha1-antitrypsin, which is a risk factor for the development of end-stage liver disease, fails to induce the UPR. As such, the efficiency by which the misfolded Z variant is subjected to ERAD must rely, at least in part, on the low basal levels of ERManI. Considering this potential vulnerability, we asked whether genetic variations in the human ERManI gene might influence the rate at which ZZ patients develop end-stage liver disease. In support of this hypothesis, homozygosity for a single nucleotide polymorphism (SNP) (rs4567(A)) in the 3'UPR of ERManI coincides with an accelerated onset of the end-stage liver disease. Functional studies demonstrated that the SNP generates a conditional hypomorphic allele that suppresses the translation of ERManI in response to ER stress caused by the Z variant, possibly impairing the liver's capacity to deal with the rapid accumulation of misfolded alpha1-antitrypsin. Taken together, the present study demonstrates the utility of functional studies to validate the contribution of a SNP in disease pathogenesis, and introduces a novel paradigm in which a subtle defect in the multilevel regulation of gene expression can modify a classical gain-of-toxic-function disorder. The suspected contribution of microRNAs is currently under investigation.

Program/Abstract# 48

Studies on the regulatory roles of the C-terminus of Cdc48 in the ERAD pathway

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The quality control mechanism in the endoplasmic reticulum (ER) ensures proper folding of N-linked glycoproteins. Misfolded proteins are retrotranslocated into the cytosol and subsequently degraded by the proteasome. It is widely believed that Cdc48/p97 mechanically extracts ERAD substrates through the ER membrane and organizes a protein degradation complex for the efficient degradation of substrates. Cdc48/p97 interacts with various substrate-recruiting cofactors through its N-terminal domain. Recently we discovered a new protein-binding motif at the C-terminus of Cdc48/p97, which binds to most of the substrate processing cofactors, such as PNGase of high eukaryotes, and the ubiquitin fusion degradation factors, Ufd2 and Ufd3. We hypothesized that the C-terminus of Cdc48/p97 (Cdc48/p97-C) has functional and regulatory roles in ERAD. Our preliminary studies provide support for this hypothesis. We found that deletion of the C-terminal 14 amino acids from Cdc48/p97 leads to accumulation of ERAD substrates and severe growth defects in yeast cells under ER stress condition. We also found that an ufd3 mutation which blocks the interaction between Cdc48 and Ufd3 caused the depletion of the monomeric ubiquitin and acceleration of degradation of monomeric ubiquitin. We propose to further test the hypothesis by characterizing the function of Cdc48/p97-C in ERAD using biochemical, crystallographic and genetics experiments.

Supported by NIH grant GM33814 (to W.J.L.).

Program/Abstract# 49

Apical sorting by Galectin-3 dependent glycoprotein clustering

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Epithelial cells are characterized by their polarized organization based on an apical membrane that is separated from the basolateral membrane domain by tight junctions. Maintenance of this morphology is guaranteed by highly specific sorting machinery that separates lipids and proteins into different carrier populations for the apical or basolateral cell surface. We could identify the beta-galactoside binding lectin galectin-3 in lipid raft-independent apical carrier vesicles, which interacts directly with apical cargo in a glycan-dependent manner. These glycoproteins are mistargeted to the basolateral membrane in galectin-3 depleted cells dedicating a central role to this lectin in raft-independent sorting as apical receptor. The underlying mechanism is based on the formation of high molecular weight clusters in subapical endosomal compartments. Following exocytosis at the apical membrane galectin-3 is recycled back into endosomes. Together, our data suggest that glycoprotein cross-linking by galectin-3 is required for apical sorting of non-raft associated cargo.

Program/Abstract# 50

The cytoplasmic PNGase-dependent ERAD pathway and free oligosaccharides (fOSs) in *Saccharomyces cerevisiae*

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ERAD (ER-associated degradation) is one of the quality control systems for newly synthesized proteins in the ER, and non-functional proteins which fail to form correct folding state are degraded in this system. Peptide:*N*-Glycanase (PNGase) is a deglycosylating enzyme which releases *N*-glycans from glycoproteins/glycopeptides. Previously we have identified a mutant of plant toxin protein, RTA (ricin toxin A chain-mutant) as the first example ERAD substrates which degrades in a Png1 (the cytoplasmic PNGase in *S. cerevisiae*)(1, 2). Recently we have developed a new genetic device to assay the Png1-dependent ERAD pathway using the new model protein designated as RTL. In this symposium we will summarize the results of ERAD assay using RTL and various ERAD-related mutants, and will provide new insight into the similarity/difference between Png1-dependent ERAD pathway and the ones previously characterized. Upon PNGase reaction, free oligosaccharides (fOSs) are formed and catabolized in a pathway which remains poorly understood. We have recently established a method for isolation and quantitation of fOSs from the cytosol of *S. cerevisiae*, and our results indicated that structures and amount of fOSs can be a good marker to monitor the ERAD event in this organism. Formation and degradation pathway of fOSs in yeast will be discussed.

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Program/Abstract# 51

Structural basis of the molecular recognition by ERGIC-53 involved in the glycoprotein traffic in the cell

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ERGIC-53 is an intracellular animal lectin that acts as a cargo receptor in the anterograde transport of glycoproteins from the endoplasmic reticulum to the *cis*-Golgi complex. The ERGIC-53-mediated glycoprotein transport is regulated through the interaction with MCFD2, which is an EF-hand calcium-binding protein. Some gene mutations of either ERGIC-53 or MCFD2 result in the combined deficiency of the blood coagulation factors V and VIII. However the molecular mechanisms of the glycoprotein transport mediated by the ERGIC-53 and MCFD2 have not fully understood. Here we reported the structural studies of complexes of ERGIC-53 with oligosaccharide and MCFD2.

We crystallized the complex between ERGIC-53 and MCFD2 and solved their structure to 2.0 Å resolution by molecular replacement. The crystal structure suggests that ERGIC-53 has two possible binding sites for MCFD2. Then we performed the analytical centrifugation and this experiment indicated that ERGIC-53 binds MCFD2 with a 1 : 1 stoichiometry. To further understand of molecular recognition of ERGIC-53 we analyzed the interaction of ERGIC-53 with MCFD2 and oligosaccharide using NMR spectroscopy. The chemical shift perturbation data revealed that MCFD2 binds the loops connecting the β-strands of β-sandwich structure of ERGIC-53, while the sugar-binding site of ERGIC-53 locates on a concave face on the opposite surface. We also found that the structure of MCFD2 complexed with ERGIC-53 is distinct from the free MCFD2, suggesting that MCFD2 could bind the polypeptide portion of the glycoprotein through the structural alteration upon binding to ERGIC-53. Based on these data, structural basis of the intracellular traffic of glycoproteins mediated by ERGIC-53 will be discussed.

Program/Abstract# 52

Concepts in N-linked protein glycosylation

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N-linked protein glycosylation is the most frequent protein modification in eukaryotic cells. This process initiates at the membrane of the Endoplasmic Reticulum, where an oligosaccharide, Man5GlcNAc$_2$, is assembled on the lipid carrier, dolichylpyrophosphate, translocated across the membrane and completed to Glc$_3$Man$_9$glcNAc$_2$. This oligosaccharide is then transferred to selected asparagine residues of nascent polypeptide chains. The model system *Saccharomyces cerevisiae* was used to characterize this complex pathway at a molecular level. N-linked protein glycosylation does also take place in archaea and in bacteria. The recently discovered N-linked protein glycosylation process in *Campylobacter jejuni* was transferred into *Escherichia coli*, enabling a genetic and biochemical analysis of the prokaryotic pathway. The high sequence similarity of the bacterial oligosaccharyltransferase with one subunit of the eukaryotic enzyme, the very similar protein acceptor sequence as well as the finding that oligosaccharides linked to isoprenoid lipids serve as substrates in the reactions suggest that the bacterial and the eukaryotic N-linked protein glycosylation are homologous processes. In contrast to the bacterial process, N-linked glycosylation in higher eukaryotes occurs before folding of the protein, enabling the use of the N-linked glycan as a general signal that reflects the folding status of the protein. The direct comparison of the homologous process in pro- and eukaryotes made it possible to formulate hypotheses regarding the eukaryote-specific components of the pathway. In particular, the role of eukaryote-specific subunits of the oligosaccharyltransferase complex can be addressed.

Program/Abstract# 53

Impaired expression of TLR in Trypanosoma cruzi infected dendritic cells from galectin 3 defficient mice

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Galectins are a family of carbohydrate binding proteins, with specificity towards b-galactosides that trigger a large variety of biological signals upon interaction and cross-linking ligands. Have been described as danger signals because they can recognize glycoconjugates on the surface of pathogens and regulate the immune response, controlling the Th1/Th2 balance, T cell apoptosis, cell adhesion and migration. Is well known that T. cruzi is recognized by TLR2 and TLR9, and maybe TLR4, this recognition leading to an efficient cytokine production. We have shown that DCs from Gal 3 KO mice are not producing proinflammatory cytokines in response to T. cruzi and the question arises: Do TLRs work properly in Gal 3 KO mice? Here we present evidence that upon infection, infected DCs from Gal 3 KO mice exhibit dramatic impairment on the surface TLR expression compared with wild type infected DCs. In particular the expression of TLR1 after infection was almost suppressed. The relevance of these findings will be discussed on the context of the experimental infection.

Program/Abstract# 54

Role of galectins in the innate immune response to leishmaniasis

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Galectins are a class of novel carbohydrate (beta-galactoside) binding animal lectins that have been shown to be involved in key events of the immune and inflammatory response. The role of galectins in the context of leishmaniasis, however, remains undefined. We have previously shown that lack of galectin-3 (chimera-type galectin) affected the leukocyte recruitment in infected lungs during infection by *Streptococcus pneumoniae*. Using galectin-3 Knock-Out mice, we have been able to detect a similar defect in leukocyte especially in neutrophil recruitment in the case of cutaneous infection by *Leishmaniamajor*. Interestingly, the lack of availability of these innate immune cells seems to alter the course of *L. major*-induced pathogenesis as a result of a modulation in the development of immunity towards the parasite. This is in addition to our work that showed the involvement of galectin-3 in the species-specific recognition of Leishmania. Our current work involves a way of characterizing the importance of host galectins in regulating the early events of *L. major* infection and in determining the nature of response that lead to the outcome of disease.

Program/Abstract# 55

Rat CD24 is one of major poly-N-acetyllactosamine-carrying glycoproteins in PC12 cells and rat bone marrow, but not in PC12D cells and rat brain

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PC12D cells, a subline of PC12 pheochromocytoma cells, extend neurites faster than PC12 cells in response to nerve

growth factor (NGF) and cyclic AMP. In addition, PC12D cells differ also morphologically from PC12 cells, being flat in shape and having extended short processes without any stimulation. Previously we showed that the length and content of poly-N-acetyllactosamine chains obtained from the membrane fraction differed significantly between PC12 and PC12D, and also that NGF stimulation decreased the content of poly-N-acetyllactosamine chains of PC12 cells, but had no effect on PC12D cells. To characterize poly-N-acetyllactosamine -carrying glycoproteins (PL-GPs), the membrane fractions were isolated from PC12 cells by using phase separation with Triton X-114, and then by DSA-lectin column chromatography. The isolated PL-GPs were analyzed by SDS-PAGE and fluorography as well as the susceptibility to endo-β-galactosidase. The treatment of endo-β-galactosidase caused the one of major PL-GP with a molecular weight of around 62 kDa to convert into a GP with a molecular weight of 35 kDa. As the amino acid sequence of 62 kDa PL-GP containing fraction resembled that of rat CD24, we confirmed whether the smear band around 62 kDa PL-GP on SDS-PAGE correspond with rat CD24. Immunoblot analysis and susceptibility to endo-β-galactosidase treatment showed that the rat CD24 in PC12 and bone marrow cells turned out the major PL-GP with a molecular weight of around 62 kDa. To our surprise, the CD24 in brain cells had no poly-N-acetyllactosamine chains at all.

Program/Abstract# 56

Structural characterization and bioactivities of hybrid Carrageenan-like sulfated galactan from Red Alga Furcellaria lumbricalis

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The red alga, *Furcellaria lumbricalis* from the coast of the Prince Edward Island (PEI) in Atlantic Canada, was extracted with hot water and fractionated with KCl to obtain a carrageenan-like polysaccharide. The polysaccharide was further purified on ion-exchange and gel-permeation chromatography to yield a charge and size uniform fraction FB1 with an average molecular weight of 428 kD determined by high-performance gel permeation chromatography (HPGPC). Sequences of sulfated and non-sulfated oligosaccharides from a two-step acid hydrolysis was determined by electrospray ionization collision induced dissociation tandem mass spectrometry (ESI-CID-MS/MS) technique. On the basis of chemical and spectroscopic analysis, FB1 was characterized to be composed of 1,4-3,6-AnGal (40%), 1,3-4-sulfated-Gal (30%), 1,3-Gal (20%), 1,4-Gal (8%) and 1,4-3-O-methyl-Gal (2.1%), which makes it be a novel hybrid sulfated galactan. Based on the detail sequences analysis of oligosaccharides, FB1 was further characterized as a linear hybrid carrageenan-like sulfated galactan with two major blocks: κ/β-carrageenan units -$[G4S\text{-}A\text{-}G\text{-}A]_n$- (75%) and a non-sulfated galactan units -$[G\text{-}D]_n$- (10%), and three small building blocks of [G4S-D], [G-D3Me] and [G4S-D3Me] distributed in the main polysaccharide chain. In addition, the sulfated oligosaccharides prepared from FB1 were shown to possess immunomudulatory activity and inhibition against BACE *in vitro*. Fraction M6 appears to be the most potent immunomodulatory oligosaccharide, and could be a candidate for further study.

Program/Abstract# 57

Synthesis of potential DNA bisintercalators based on sugar skelecton

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Intercalators are among the most important group of small molecule that interacts reversibly with the DNA double helix. [1] Bisintercalators have two potential intercalating ring systems connected by linkers which can vary in length and rigidity. In recent years, the synthesis of various bisintercalators has been studied extensively for their higher DNA-binding capacity, slower dissociation rates and substantial sequence selectivity compared with monointercalators. [2]

Monosaccharides have been introduced and validated as biologically relevant scaffolds. Advantages of using saccharides are that they display a high density of functional groups, are available as single enantiomers and contain multiple sites for attachment of recognition groups.[3]

Here glucose, 2-aminoglucose and glucuronic acid were used as the carbohydrate scaffolds, coupling with different chromophores including quinoline, acridine, indol[3,2-b]

quinoline ring, indole and purine and then coupled with different linker to get the compounds.

The interactions of these compounds with calf thymus DNA (CT-DNA) have been investigated with UV-absorption, fluorescence spectroscopy, DNA competitive binding with ethidium bromide and viscosity measurements. The experimental results indicate these compounds show efficient binding toward CT-DNA through intercalation. And the glucose group of compound can regulate their interactions with the DNA duplex. [4]

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Program/Abstract# 58

Solid-phase synthesis of amino-functionalized glycoconjugates for attachment to solid surfaces and proteins

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Carbohydrates and their derivatives are involved in numerous processes in the body, for example pathogenesis. To study important and disease-related carbohydrate structures pure and well-characterized glycoconjugates are needed. Isolation from natural sources is difficult and time consuming and for that reason straightforward synthetic strategies for glycoconjugates are required. To reach this goal we develop methods for solid-phase synthesis monitored with gel-phase 19F spectroscopy using fluorinated linkers and protective groups (Spjut S. *et al*., *Eur. J. Org. Chem*. **2009**, 349–357, Wallner F.K. *et al*., *Org. Biomol. Chem*. **2005**, 3, 309–315). Recently we have applied a carbamate-based strategy to attach a spacer carrying an amino group to a fluorinated Wang linker for synthesis of amino-functionalized glycoconjugates using thioglycoside donors with fluorinated protective groups. Cleavage from the solid support was performed with trifluoroacetic acid and subsequent protective group removal gave the target compound. The terminal amine was conjugated with didecyl squarate and this derivative can be attached to various proteins and solid surfaces carrying primary or secondary amines. To evaluate this methodology we have immobilized glycoconjugates in amino-functionalized microtiter plates and successfully probed them with lectin.

Program/Abstract# 59

Probing the structures and interactions of glucans with proteins by electrospray mass spectrometry and carbohydrate microarrays

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Glucans are homopolymers of D-glucose with differing linkages. β-Glucan polysaccharides are of increasing interest due to their properties as modulators of the immune system and as targets for immunotherapy of fungal infections. We are developing microarrays of oligosaccharides derived from glucan polysaccharides of various fungal, bacterial and plant origins for mapping the determinants on polysaccharides recognized by glucan-binding proteins. Examples have been a key receptor of the innate immune system to fungal pathogens, Dectin-1 (1), and antibodies elicited by glucan-based vaccines (2). Glucan recognition systems differ in their requirements for specific glucose-linkages and chain lengths. In this presentation we will describe a strategy using negative-ion electrospray tandem mass spectrometry with collision-induced dissociation for high-sensitivity linkage determination of the major types of neutral linear glucan oligosaccharides and these include the 1–2, 1–3, 1–4, and 1–6 linkages. The presence of glycosidic C-type cleavage can be used to define linear sequences, whereas the different and unique cross-ring A-type fragmentations can be used for linkage assignments. Microarray analyses of the glucan oligosaccharides as neoglycolipid probes (3) with selected glucan-binding proteins and assignments of their specificities will also be described.

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Program/Abstract# 60

Synthesis of glycosaminoglycan oligosaccharides equipped with fluorogenic groups for FRET

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A number of efforts have been made to detect the enzymatic activity using artificial substrates. Many types of specific enzymes are deposited during the growth of diseases. Carbohydrates are often degraded by the disease specific enzymes, thus the detection of the enzyme would enable to find the disease in early stage. We paid attention

to glycosaminoglycans, glycan part of proteoglycan, which abundantly exist in the tissues of the animals. The glycosaminoglycan specific enzymes deeply relate to proliferation, regulation of neuronal growth, and cell recognition. The facile structures of the glycosaminoglycans including the stereochemistry of the sugar residues, length of the glycan chain and the positions of the sulfate groups reflect the specificity of the enzymes. We synthesized some glycosaminoglycan oligosaccharides which could be cleaved by specific glycosidases. Highly sensitive fluorogenic groups on the substrates are useful for the detection in a micromolar level.

We prepared the glycosaminoglycan substrates equipped with the chromophore donor (X) and the fluorophore acceptor (Y) at the reducing and non-reducing terminals, respectively. A selected pair of the fluorogenically bifunctional groups of the oligosaccharide can show FRET (Fluorescence Resonance Energy Transfer). After the enzymatic cleavage of the substrate, the two fluorogenic groups are separated, and the FRET could not be possible. This strategy will enable to detect the disease specific enzymes.

Program/Abstract# 61

Synthesis of primers for biosynthesis of proteoglycan

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Glycosaminoglycan (GAG), a glycan part of proteoglycan (PG), covalently binds to the core protein at the reducing terminal. GAG is composed of the linkage tetrasaccharide and the repeating disaccharide region. The latter is classified into heparan and chondroitin families based on the type of the hexosamine residues. The sulfation patterns on hydroxyl and amino groups complicate the structure of PG and GAG. PG and GAG show characteristic biological properties depending on the structures in a molecular level. It is suggested that the clustered GAGs in the PG molecule would amplify the biological activities. However, it is difficult to determine the relationship between the detail structure and the biological activity due to the heterogeneity of naturally occurring PG.

In order to clarify the ambiguousness we synthesized series of small PG which can be primers for enzymatic GAG elongation. The small PG is composed of unprotected linkage trisaccharide and tetrapeptide unit (Gal-Gal-Xyl-SGSG) equipped with dansyl group (DNS) at the N-terminal. One by one couplings of the N-terminal of the unit to the C-terminal of the oligomer were successfully achieved by the help of DMT-MM as a promoter even in water. We finally synthesized the corresponding tetramer, DNS-(Gal-Gal-Xyl-SGSG)4. Enzymatic glycan elongation to the primer composed of the oligomer of the glycosyl peptide unit would be able to construct a homogeneous PG having GAG at regular intervals on the core peptide.

Program/Abstract# 62

Design and synthesis of the potential transition state analog inhibitors of glycosyltransferases

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Glycosyltransferases (GT's) represent an important group of enzymes involved in the biosynthesis of *N*- and *O*-linked complex oligosaccharides of glycoproteins. They cause significant structural variations in biological systems and changes in protein glycosylation are also early indicators of cellular changes in many diseases.

This contribution is aimed at developing of the transition state (TS) analog inhibitors of the GT's. The structure of the TS model for the catalytic reaction of glycosyltransferase GnT-I determined using a hybrid QM(DFT)/MM method [1] was used to design TS analogue. Herewith we introduce the synthesis of four precursors, namely benzyl 2-thio-6-*O*-*tert*-butyldimethylsilyl-α-D-fructofuranoside 1-diethylphosphate (**1**), its β-anomer (**2**), and their ethyl 2-thio analogues (α-anomer **3** and β-anomer **4**).

Starting from benzyl or ethyl 2-thio-α- or β-D-fructofuranosides, respectively [2], sequential protection at position C-6 with *tert*-butyldimethylsilyl group, at C-1 with dimethoxytrityl group, at C-3 and C-4 with acetyl, followed by detritylation afforded nucleophiles having a free OH-group at C-1 [3]. These were coupled with diethyl chlorophosphate to give fructofuranoside 1-diethylphosphates. The desired precursors **1**–**4** were finally obtained by usual deacetylation.

Acknowledgements
This work was supported by the grants APVV-51-004204 and VEGA No. 2/0128/08.

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Program/Abstract# 63

Synthesis of KDO-human serum albumin conjugate

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2-Keto-3-deoxy-α-D-*manno*-octulosonic acid (Kdo) is a component of lipopolysaccharide (LPS) or lipooligosaccharide (LOS) produced by gram-negative bacteria. Kdo, which is the reducing end of the intact core oligosaccharide (OS), α-links the core OS to the Lipid A. Yamasaki *et. al.*

reported that normal human sera (NHS) contain bactericidal antibodies specific for a site close to the core OS expressed on gonococcal LOS and such core OS or a nearby site could be utilized as possible targets for vaccine development against microbial infections [1]. Toward the development of conjugate vaccine, we have been synthesizing a part of inner core OS of LOS [2] and its protein conjugates. Recently, we reported that an α-selective anomeric acylation of the Kdo drivative with an isocyanate linker having an appropriate function to conjugate a carrier protein [3]. Here we describe the synthesis of a Kdo derivative-HSA conjugate using our O-acylation method. The conjugation was based on formation of amide linkages between the Kdo residue and a dicarboxylic acid linker bound to the carrier protein. This procedure yielded high molecular mass conjugates, revealed by MALDI-TOF MS, SDS/PAGE, and protein. The number of Kdo residue per protein was calculated from the molecular mass of the conjugate, the carrier protein.

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Program/Abstract# 64

Aminoacyl saccharide as organocatalysts for asymetric aldol reaction

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Proline acts as the efficient catalyst for the asymmetric aldol reaction in organic medium, but is not effective for the reaction in the presence of water.

During the search for improved catalyst, proline derivatives possessing hydrophobic auxiliaries have been reported as efficient catalysts for the reaction in aqueous medium.

However, the incoming water molecule to generate the transition state was reported to be critical to accelerate the aqueous aldol reaction[1], and the reaction mechanism suggested that hydrophilic auxiliaries might be better to control the incoming water molecule.

In order to search the effect of carbohydrate moiety as hydrophilic auxiliaries on the aldol catalysts, saccharide moiety was conjugated with amino acid residue to prepare aminoacyl saccharides as organocatalysts[2]. Amongst the aminoacyl saccharides prepared, prolinamido-glycoside for the reaction in the presence of water catalysts were effective with respect to yields and enantiomeric excesses. The efficiency was achieved up to 80% yield and 90%ee by the use of methyl 2-deoxy-2-prolinamido-α-D-glucopyranoside in the reaction of water miscible substrates. Thus, the prolinamide-glycoside catalysts were successfully applied for the synthesis of ketopentose from dihydroxyacetone and glyceraldehyde. The structure-catalytic ability relationship study of aminoacyl-saccharides prolinamido-glycosides proved the effects of hydrophilic auxiliary on the catalyst for the asymmetric aldol reaction.

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Program/Abstract# 65

Overexpression of MAN2C1 boosts ERAD and leads to accumulation of free oligomannosides

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One-third of newly synthesized proteins are rapidly degraded via the proteasomal complex. Major part of them are constituted by N-glycoproteins for which the quality control was inefficient. After retrotranslocation in the cytosol, glycoproteins are deglycosylated, ubiquitinylated before degraded by the proteasomal complex. The released free oligosaccharides are sequentially trimmed by the beta endo-Nacetylglucosaminidase and the alpha-mannosidase MAN2C1 before imported onto the lysosomes. Even if the sequential events of ERAD of N-glycoproteins are well described, the regulation of this catabolism remains poorly understood. One explanation could be attributed to the different intracellular compartments involved in this catabolism, endoplasmic reticulum, cytosol and lysosomes. In this study, we analyzed the biochemical effects of the overexpression of the human MAN2C1 in HeLa cells (NAM cells). We demonstrated that a high expression level of Man2C1 was correlated to the accumulation of small free oligomannosides ($Man_{2\text{-}3}GlcNAc_1$) in the cytosol. In addition, overexpression of MAN2C1 was correlated to an increasing rate of ERAD since level of ubiquitinylated proteins was higher in NAM cells cells in the presence of proteasomal inhibitors. Since ERAD represents the major

source of soluble free oligomannosides, this study would open new perspectives about the regulation of catabolism of glycoproteins.

Program/Abstract# 66

Proteomic analysis of GNA and RSA binding proteins in the pea aphid

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The *Galanthus nivalis* agglutinin (GNA) as well as the *Rhizoctonia solani* agglutinin (RSA) have proven to be toxic to the pea aphid *Acyrthosiphon pisum.* In an attempt to unravel the mode of action of these lectins, a proteomics approach was used to investigate which pea aphid proteins bind to an affinity column with immobilized GNA, a lectin that specifically interacts with high mannose N-glycans, or RSA, a lectin that specifically targets galactosylated glycans. Purified proteins were directly in-solution digested using trypsin and the resulting peptides identified by LC-MS/MS analysis using an electrospray ionization-ion trap mass spectrometer. Using the Mascot search algorithm and the Swissprot database (version 14.9) we were able to identify many proteins in the *A. pisum* samples. These proteins were then categorized into different groups based on their function in digestion, metabolism, transport, protein biosynthesis, stress response, proteolysis, etc. Using the Scan Prosite tool available at http://www.expasy.org, motifs for N-glycosylation consensus sites were analyzed. The current investigation contributes to our understanding of these glycosylated *A. pisum* proteins that can specifically interact with GNA and RSA, and may contribute to the insecticidal activity of the lectins. In addition, this investigation yields interesting information regarding the glycome of the pea aphid.

Program/Abstract# 67

Transcript analysis of glycan-related genes in human embryonic stem cells

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We have previously developed a qRT-PCR-based transcript profiling approach to analyze the expression of all glycan-related genes in mouse cells and tissues. Transcript profiles were compared with glycan structures produced during differentiation of murine embryonic stem cells (mESCs) to embryoid bodies (EBs) and extra-embryonic endoderm (ExE) to better understand the changes in glycosylation that occur during stem cell differentiation. In particular, transcripts and glycans were compared for mESCs, EBs, and ExEs and numerous correlated changes were demonstrated, indicating transcriptional regulation of glycan-related structures during mouse ESC differentiation.

We are currently examining transcript levels during human ESC differentiation to compare with the murine transcript profiles and glycan structural data. Variability in glycan-related transcripts was examined in several human ESC lines, as well as during time course studies of differentiation toward mesoderm (Meso) and definitive endoderm (DE) lineages. Transcript profiles indicated fewer changes in glycan-related transcripts during human ESC differentiation, but significant up-regulation of protein α2,8-polysialyltransferase transcripts was similar to differentiating mouse ESCs. In mESCs, ST8Sia2 was up-regulated during differentiation to EBs or EXEs, consistent with increases in cell surface polysialylated glycans in differentiated cells. In contrast, differentiation of human ESC to Meso or DE lineages resulted in up-regulation of ST8Sia4 in correlation with increases in cell surface polysialylated structures. Additional transcript profiles and comparison with corresponding glycan structures will be presented. (Supported by NIH grant RR018502).

Program/Abstract# 68

Ternary supra-molecular complex of oligosaccharyltransferase, Sec61 complex and ribosomes

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The oligosaccharyltransferase (OT) transfers high mannose type N-glycans to nascent chains that are translated by membrane-bound ribosomes and translocated into the endoplasmic reticulum (ER) through the translocon channel formed by the Sec61 complex. Importantly, the translocation and N-glycosylation processes are co-translational events. Structural studies on the co-translational translocation have revealed that the nascent chain-exiting site of ribosomes is connected to the translocon channel to ensure that the nascent chains are translocated into the ER lumen or membrane. We have previously reported that in yeast microsomes OT is chemically cross-linked to components of the Sec61

complex. This observation led us to hypothesize that OT may be adjacent to the translocon channel and form a supra-molecular complex with the Sec61 complex and ribosomes. To test this hypothesis we separately purified OT and the Sec61 complex from yeast and analyzed binding of OT and the Sec61 complex to purified yeast ribosomes by co-sedimentation analysis and by electron microscopy. Interestingly we found that functionally active OT forms a ternary complex with the Sec61 complex and ribosomes. We further found that OT directly binds to the ribosomes with a stoichiometry of approximately 1 to 1, and that the binding occurs at a position near the Sec61 complex-binding site of ribosomes. Based upon existing data and our new findings, we propose that co-translational translocation and N-glycosylation of nascent chains are mediated by a ternary supra-molecular complex consisting of OT, the Sec61 complex and ribosomes. (Supported by NIH Grants GM33185)

Program/Abstract# 69

Monitoring anti-angiogenic action of Tunicamycin by Raman spectroscopy

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N-glycans are important for glycoprotein folding. Accumulation of un-folded proteins results in loss of cellular integrity and the development of “ER stress”. To study the physiological significance of the “ER stress” in capillary endothelial cells, we have used Tunicamycin, an 840 Da glucosamine-containing pyrimidine nucleoside, and a competitive inhibitor of *N*-acetylglucosaminyl 1-phosphotransferase. This enzyme prevents the synthesis of $Glc_3Man_9GlcNAc_2$-PP-Dol, a precursor of asparagine-linked (N-linked) oligosaccharides. This causes cell cycle arrest and cell destruction by inducing apoptosis. To evaluate Tunicamycin-induced protein structure alteration in capillary endothelial cells, we have used Raman spectroscopy of cells cultured for 3 and 12 hr with and without Tunicamycin. The spectra (collected at room temperature, at integration 400, average 1 and box 10) displayed Raman bands at 1672, 1684 and 1694 cm^{-1}, which are characteristic of proteins and originate from C=O stretching vibrations of the Amide I of monosubstituted amides. In the presence of Tunicamycin, these bands decreased in area as follows: at 1672 cm^{-1} by 41.85 and 55.39% at 3 and 12 hrs, respectively; at 1684 cm^{-1} by 20.63 and 40.08% at 3 and 12 hrs, respectively; and at 1994 cm^{-1} by 33.33 and 32.92% also at 3 and 12 hrs, respectively. These results show that in the presence of Tunicamycin, newly synthesized protein chains fail to arrange properly into their final structures, and demonstrate the reliability of Raman spectroscopy to follow anti-angiogenesis *in vitro*. Supported in part by a Susan G. Komen for the Cure grant BCTR0600582.

Program/Abstract# 70

Evolutionary trends in protein N-glycosylation

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Morphogenesis is dependent on secreted factors and transmembrane receptors to fashion the body plan, a feature of metazoan evolution likely to have been under intense selective pressures. Cytokine receptors differ in NXS/T number, which allows for their differential regulation by metabolic flux into UDP-GlcNAc and N-glycan branching (Lau *et al* ***Cell*** 128, 123, 2007). This suggests that evolutionary trends in N-glycan number for individual glycoproteins and conditional regulation of sugar nucleotides and Golgi enzymes may allow adaptation at a systems level. To begin exploring this possibility, we compared the NXS/T sites in extracellular proteins for species ranging from yeast to human. We observed conservation of site densities, but at the same time, a reduced probability of ectopic sites. With higher NXS/T number, glycoproteins display slower evolution rates, possibly reflecting purifying-selection for stable protein folds. An evolutionary decrease in genomic A base content and consequently Asn, reduces the probability of ectopic NXS/T sequences from yeast to vertebrates. A comparison of secreted and non-secreted proteins in the human genome suggests glycoproteins are under selection and the rest of the genome has been carried along. Metazoan evolution has seen an expansion of genes encoding Golgi enzymes for N-glycan modification and animal lectins. Our study provides evidence that in addition to their ancient role in protein folding and stability, high and low N-glycan number cooperates with Golgi modification to differentially regulate glycoprotein trafficking.

Program/Abstract# 71

Glycosylation in a Unified approach to discover the cellular origins of disease

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From the discovery of DNA to the sequencing of the human genome, the template-dependent formation of

biological molecules from gene to RNA and protein has been the central tenet of biology. Yet the origins of many grievous diseases remain a mystery. Diabetes, autism, allergy, Alzheimer's disease, Lou Gehrig's disease, multiple sclerosis, Parkinson's disease, and many others continue to evade understanding. Expectation that defined variation in the DNA blueprint would serve to pinpoint even multigenic causes of these diseases remains unfulfilled. The genetic parts list appears insufficient to explain the pathogenic origins of these diseases. While the genome provides the framework and basic instructions upon which the cell develops and operates, the full complexity of cellular life cannot be encoded in nucleic acid sequences. Other factors are seemingly involved. This laboratory has observed that the molecular and cellular origins of Type 2 diabetes, inflammatory and autoimmune diseases, and the lethal coagulopathy of sepsis include template-independent biological processes that cannot be detected by current genomic or proteomic technologies. Of the four fundamental macromolecular components of all cells, and which include the nucleic acids and proteins, the lipids and glycans are not directly encoded by the genome and yet their modulation participates in disease pathogenesis. Such findings reveal why a renewed focus on the cell as the fundamental unit of life in an era of cell biology will lead to further resolution to disease origins, and thus provide mechanistic insights needed to perceive and effectively intervene with new approaches to diagnosis, prevention, and cure.

Program/Abstract# 72

Role of “Glycan cycle” in disease; lessons from functional glycomics

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Over half of all known proteins in eukaryotes are glycosylated and the glycans are implicated in various diseases such as infectious diseases, cancer, degenerative nerve and muscle diseases and life-style related diseases. Our group has been interested in branched N-glycans produced by glycosyltransferases, GnT-III, GnT-V, and Fut 8 whose enzymatic products are bisecting GlcNAc, beta 1,6 GlcNAc branch and core fucose, respectively. Those glycans markedly affect the functions and structures of glycoproteins. We have found that the target proteins of these enzymes are mostly cell surface receptors or adhesion molecules. Fut 8 KO mice are semi-lethal and survivors develop emphysema-like changes due to the lack of core fucose of TGF-beta receptor, resulting in dysfunction of TGF beta signaling and activation of MMP (matrix metalloproteinase) genes. Activation of MMPs degrades the alveolar walls and leads to emphysema of the lung. Core fucose is also important for cancer biomarker for hepatoma and pancreatic cancer and also play key roles in antibody therapy against cancer. GnT-III and GnT-V act competitively with each other in terms of cancer metastasis and cell adhesion via E-cadherin and integrins as target glycoproteins of the above glycosyltransferases. In order to elucidate the integrative functional roles of glycans, it is essential to understand the dynamic changes of glycans, which has not been done yet even though many data are available in terms of changes of nucleotide sugars and their transporters, glycosyltransferases and glycan structures under physiological and pathological conditions. This lecture will also describe the concept of “Glycan cycle”, which comprises a biochemical and molecular network as a functional unit which may play a pivotal role in the cell surface receptor signaling by glycans.

Program/Abstract# 73

Absence of polysialic acid - an unfavorable prognostic marker of advanced stage neuroblastoma

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Polysialic acid is the capsular polysaccharide of some neurotrophic bacteria, such as group B meningococci and *E. coli* K1. In eukaryotes polysialic acid units are found in the carbohydrate units of embryonic neural cell adhesion molecule NCAM. Polysialic acid is a neural crest stem cell marker, and it, as well as NCAM, have been detected in some malignant tumors with high metastatic activity and unfavorable prognosis. The diagnostic and prognostic value of polysialic acid in neuroblastoma is, however, unclear. For the detection of polysialic acid we have developed a method employing catalytically inactive endosialidase, which binds to polysialic acid but does not cleave it. Inactive endosialidase fused to green fluorescent protein via spacer was found to be an effective and sensitive method for the detection of polysialic acid. A tumor tissue microarray (TMA) of 36 paraffin-embedded neuroblastoma samples was utilized to detect polysialic acid expression with the polysialic acid-binding fusion protein, and polysialic acid expression was compared with clinical stage, age, MYCN amplification status, histology (INPC), and proliferation index (PI). Polysialic acid-positive neuroblastoma patients had more often metastases at diagnosis, and polysialic acid expression associated with advanced disease ($P=0.047$). However,

absence of polysialic acid-expressing tumor cells in TMA samples was a strong unfavorable prognostic factor for overall survival in advanced disease ($P = 0.0004$), especially when MYCN was not amplified. Polysialic acid-expressing bone marrow metastases were easily detected in smears, aspirates and biopsies. Polysialic acid appears to be a clinically significant molecular marker in neuroblastoma, with potential value in neuroblastoma risk stratification.

Program/Abstract# 74

Remodeling of N-glycosylation pathway of the methylotrophic yeast Hansenula polymorpha: evaluation of the ALG3 deletion strain blocked in the lipid-linked oligosaccharide assembly as a host for the production of therapeutic glycoproteins

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The thermotolearant methylotropic yeast *Hansenula polymorpha* has some advantages over the traditional yeast *Saccharomyces cerevisiae* in the production of recombinant glycoproteins for human therapeutic use, such as less hypermannosylation and lack of highly immunogenic terminal α-1,3-linked mannose residues. As a first step toward humanizing *H. polymorphaN*-glycosylation pathway, we developed the *H. polymorpha och1Δ* mutant strain, having a defect in the outer chain initiation on the core oligosaccharide $Man_8GlcNAc_2$, with the targeted expression of *Aspergillus saitoi*α-1,2-mannosidase in the ER. The engineered *H. polymorpha och1Δ* strain produced the human high mannose-type $Man_5GlcNAc_2$ oligosaccharide as a major *N*-glycan. As an alternative approach, we carried out the remodeling of core oligosaccharide assembly pathway by additional deletion of the*H. polymorphaALG3* gene, encoding a dolichyl-phosphate-mannose dependent α-1,3-mannosyltransferase. The engineered double deletion (*Hpalg3ΔHpoch1Δ*) strain expressing *A. saitoi*α-1,2-mannosidase generated mainly the trimannosyl-core form glycan ($Man_3GlcNAc_2$), an intermediate for further maturation to human-like complex *N*-glycans.We have performed subsequent modification of *H. polymorpha* glycosylation pathway to synthesize the complex-type *N*-glycans with a terminal *N*-acetyl glucosamine in the glycoengineered *ΔHpoch1* and*ΔHpoch1ΔHpalg3* strains, respectively. Several combinatorial synthetic leaders were constructed for the localized expression of active human β-1,2*N*-acetyl glucosaminyl transferase I at the Golgi membrane, and the production of complex-type glycans with mono-antennary *N*-acetyl glucosamine was analyzed by a capillary electrophoresis of ATPS-labeled cell wall glycans. The comparative analysis strongly suggested that the *ΔHpoch1* singledeletion strain would be a more suitable host for further manipulation toward human complex-type *N*-glycansthan the*ΔHpoch1ΔHpalg3* double deletion strain in the aspects of the glycosylation site occupancy and the byproduct Hex_6GlcNAcs formation.

Program/Abstract# 75

Chemical protein glycosylation: a new approach to protein stabilization

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Protein pharmaceuticals have outstanding potential in the cure and prevention of diseases and have already substantially expanded the field of molecular pharmacology. Unfortunately, proteins (and peptides) frequently display substantial chemical and physical instabilities hampering their success as drugs. Detrimental stresses encountered during manufacturing, storage, delivery, and other pharmaceutically relevant processes, frequently alter the chemical composition and the three-dimensional structure of proteins thus negatively impacting their therapeutic efficacy and giving rise to potential safety hazards for patients (*e.g.*, immune reactions triggered by protein aggregates). This has prompted an intense search for novel strategies to stabilize pharmaceutical proteins. Due to the well known effect of glycans in increasing the overall stability of glycoproteins, rational manipulation of the glycosylation parameters through glycoengineering could become a promising approach to improve both the *in vitro* and *in vivo* stability of protein-based pharmaceuticals. The intent of this presentation is to survey the different physicochemical instabilities displayed by proteins during their pharmaceutical employment, how these can be prevented by glycosylation, and to discuss the currently proposed biophysical models by which glycans induce these stabilization effects.

Program/Abstract# 76

Glycan analysis of a plant-cell derived glucocerebrosidase as a tool for monitoring changes in growth condition and manufacturing

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Glucocerebrosidase (GCD) is a glycoprotein having 4 occupied glycosylation sites. It is incorporated into human macrophage cells via cell surface mannose receptors and catalyzes the hydrolysis of glucosylceramide (glucocerebro-

side, GlcCer). A mutation in the gene encoding the enzyme leads to Gaucher disease, one of the most common inherited lysosomal storage disorders. A recombinant GCD produced in carrot cell suspension cultures (prGCD) is used for enzyme replacement therapy (ERT). N-linked glycans having terminal mannose sugars are essential for efficient uptake of GCD. Glycan analysis using endoglycosidase digestion of N-glycans with PNGase F and A, coupled with exoglycosidase digestion and normal-phase HPLC separation gave us a consistent glycan profile for glycans from prGCD. The production in carrot cells is targeted to the storage vacuole, leading to a product having close to 100% terminal mannose glycan residues. The dominant glycan structure found was an N-linked core structure of two N-acetylglucosamine and three mannose sugars, substituted with an α(1-3) linked fucose and a β(1-2) linked xylose sugar. Hence, prGCD does not require additional post-production processing of the glycan structures for efficient uptake to macrophage cells. The relative ratio of the glycan structures on the harvested enzyme are sensitive to changes in growth conditions and production methods. This study summarizes over 3 years of glycan analysis of prGCD. It shows how systematic glycan analysis is a key instrument in understanding changes in growth conditions and production methods. Regular analysis is used as a tool for quality control to achieve a well characterized product for ERT. Submission of prGCD for ERT to the Food and Drug Administration will take place at the end of 2009.

Program/Abstract# 77

Glyco-engineering in plants: production of monoclonal antibodies containing homogeneously β1,4-galactosylated human-type N-glycans

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Plants are currently developing to a cost-effective, biologically safe large-scale production system for recombinant proteins. Since the majority of therapeutically relevant proteins are glycoproteins the presence of plant specific N-glycan epitopes and the absence of sialylation represent major obstacles in the use of plants as an expression platform for therapeutically relevant glycoproteins. Therefore, we developed approaches to (i) eliminate the non-human N-glycan structures and (ii) to reconstruct the peripheral N-glycan processing steps in host plants. Recently we generated plant glycosylation mutants that do not add the unwanted plant-specific β1,2-xylose and core α1,3-fucose residues to N-glycans. Monoclonal antibodies (mAbs) produced in this mutant carried predominantly N-glycans with terminal β-N-acetylglucosamine residues and were comparable in potency to CHO-produced mAbs. In the present study we went a step further by generating transgenic *Nicotiana benthamiana* plants expressing a modified version of active human β1,4-galactosyltransferase. This allowed the production of mAbs containing homogeneously β1,4-galactosylated N-glycans, which represent the major N-glycan species on human serum IgG. Trans-Golgi targeting of the β1,4-galactosyltransferase was crucial for avoiding incomplete galactosylation. Two mAbs with fully β1,4-galactosylated N-glycans displayed improved *in vitro* potency when compared with other glycoforms produced in plants or CHO cells. Thus, our results demonstrate that plant glycosylation mutants can be developed which are capable of synthesizing highly homogeneous, human-type N-glycan structures. This further increases the suitability of plant-based expression systems for the production of therapeutically relevant glycoproteins.

Program/Abstract# 78

Engineering a new class of carbohydrate-binding proteins: computationally guided mutagenesis of O-GlcNAcase

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Specific glycan (carbohydrate; CHO) structures proliferate in various cancers[1], thus are diagnostic and therapeutic targets. However, current CHO-binding proteins, *i.e.* lectins and antibodies (Abs), are limited, in that they have broad specificity, low affinity and can be toxic (lectins), or have context-dependent specificity and can be difficult to generate (Abs). Thus Abs and lectins are not always suitable as diagnostic and therapeutic reagents. To address these issues, we will generate a new class of CHO-binding proteins via the conversion of CHO-processing enzymes to high-specificity and high-affinity receptors (called "Lectenz").

Lectenz will be developed that are non-enzymatic, via sequence mutation, but have retained their specificity for CHO sequences and linkage configurations. Using crystal structure data[2], computational predictions will be used to guide the design of *in vitro* Lectenz libraries to identify residues that are essential for CHO-binding or amenable to mutagenesis. Based on this data, Lectenz libraries will be

built and screened for clones that have enhanced binding to the target CHOs. Currently, we are developing a Lectenz based on the O-GlcNAcase enzyme that binds biologically to Ser-GlcNAc and Thr-GlcNAc. Computational and *in vitro* mutagenesis data will be presented on our findings to date.

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Program/Abstract# 79

Overexpression of ST6GalNAcV, a ganglioside-specific α2,6 sialyltransferase, inhibits glioma invasivity

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Aberrant cell-surface glycosylation patterns are present on virtually all tumors and have been linked to tumor progression, metastasis, and invasivity. We have previously shown that expressing a normally quiescent, glycoprotein-specific α2,6 sialyltransferase gene (ST6Gal1) in gliomas inhibited invasivity *in vitro* and tumor formation *in vivo*. To identify other glycogene targets with therapeutic potential, we created a focused 45-mer oligonucleotide microarray platform representing the human glycotranscriptome and examined expression profiles of 10 normal human brain specimens, 10 malignant gliomas, and 7 human glioma cell lines. Among the many significant changes in glycogene expression observed, of particular interest was the observation that an additional α2,6 sialyltransferase, ST6GalNAcV, was expressed at very low levels in all glioma and glioma cell lines examined compared to normal brain. ST6GalNAcV catalyzes the formation of terminal α2,6 sialic acid linkages on gangliosides. Stable transfection of ST6GalNAcV into U373MG glioma cells produced (i) no change in α2,6-linked sialic acid-containing glycoproteins, (ii) increased expression of GM2α and GM3 gangliosides, and decreased expression of GM1b and asialo-GM1, (iii) marked inhibition of *in vitro* invasivity, and (iv) modified cellular adhesion to fibronectin and laminin. In addition, increased levels of adhesion-mediated protein tyrosine phosphorylation of HSPA8 protein that correlated with decreases in *in vitro* invasivity was observed in the transfectants. These results strongly suggest that modulation of the synthesis of specific glioma cell-surface glycosphingolipids alters invasivity in a manner that may have significant therapeutic potential.

Program/Abstract# 80

A combined method for producing homogeneous glycoproteins with eukaryotic N-glycosylation

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N-glycosylation affects the intrinsic activities of glycoproteins and the fine N-glycan structures are responsible for various important biological recognition processes. However, a major challenge in glycobiology research comes from the microheterogeneity of natural and recombinant glycoproteins, from which pure glycoforms are difficult to isolate for functional studies and biomedical applications. We present a general, two-step strategy for producing homogenous N-linked glycoproteins carrying eukaryotic N-glycans in which (i) a GlcNAc-linked glycoprotein is generated in a glycoengineered *Escherichia coli* strain and (ii) the glycan is further elaborated *in vitro* via enzymatic transglycosylation. The method thus combines the power of recombinant protein production in an *E. coli* expression system with the flexibility of *in vitro* chemoenzymatic glycan elaboration. In this way, we assembled homogeneous glycoforms of a heterologous protein representing the mammalian core pentasaccharide $Man_3GlcNAc_2$, the high-mannose type N-glycan $Man_9GlcNAc_2$, and the bi-antennary complex type N-glycan, respectively. Future research will be focused on exploring the potential of this method to assemble biologically relevant N-glycoproteins. A quick access to a variety of well-defined N-glycoproteins will facilitate structural, functional, and biomedical studies of this class of biomolecules.

Program/Abstract# 81

An update on congenital disorders of glycosylation

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N-linked glycosylation is the most frequent modification of secreted and membrane proteins in eukaryotic cells. This highly conserved process is initiated in the endoplasmic reticulum, where the Glc3Man9GlcNAc2 oligosaccharide is first assembled on the lipid carrier dolichol-pyrophosphate and then transferred to selected asparagine residues of polypeptide chains. In the recent years, several inherited

human diseases, named congenital disorders of glycosylation (CDG), have been associated with deficiencies in this pathway. Clinically, most CDG patients present with psychomotor retardation, hypotonia, cerebellar hypoplasia, hormonal disorders and stroke-like episodes. The analysis of dolichol-linked oligosaccharides in CDG cells provides a way to identify alterations of oligomannose core biosynthesis, because a defective assembly results in the accumulation of intermediate oligosaccharide structures. Using this approach, twelve forms of CDG have been identified in the last decade. The recent developments on the characterization of additional types of CDG and on novel therapeutical approaches will be discussed.

Program/Abstract# 82

Modeling glycosylation disorders in Zebrafish: new insights into pathophysiology

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While progress continues to be made towards the identification of new congenital disorders of glycosylation (CDGs), our understanding of how changes in glycosylation lead to the pathogenic consequences of these diseases remains unclear. This is due in part to the lack of appropriate animal models for many CDGs. Traditional knockout methods in mice are unsuitable in the case of CDGs since all the known defects are hypomorphic or “leaky”. In light of its genetic and experimental accessibility, we believe the generation of CDG models using the vertebrate organism zebrafish (Danio rerio) provides a useful and rapid alternative to study the developmental pathophysiology of these disorders. To this end, our laboratory has begun to characterize a morpholino-based model for CDG-Ia. Preliminary studies reveal that inhibition of PMM2 expression in zebrafish embryos results in multiple phenotypes characteristic of many CDGs (including pericardial edema and impaired motility). Molecular analysis of LLO profiles in the PMM2 morphants reveals a striking loss of the mature LLO, consistent with the robust loss of enzyme activity (4–7% of WT activity). Since impaired motility may arise due to aberrant motor neuron development, the early development of these neurons in this model was analyzed. Taking advantage of transgenic zebrafish that express GFP in primary motor neurons, we show that the pathfinding and arborization of motor neurons is substantially affected in PMM2-deficient embryos. Further analysis of this model may yield important clues into the disease process of CDG-Ia and related CDGs, with particular regard to the hypotonia and psychomotor retardation noted in these patients.

Program/Abstract# 83

The COG complex functions in trafficking of glycosyltransferases through the Golgi

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The proper glycosylation of proteins trafficking through the Golgi apparatus depends upon the Conserved Oligomeric Golgi (COG) complex[1]. Defects in COG can cause fatal congenital disorders of glycosylation (Type II CDGs) in humans[2,3]. COG is one of several evolutionary conserved protein complexes proposed to function as tethering factors, mediating the initial interaction between transport vesicles and their target membranes. COG consists of Lobe A (Cog1-4) and Lobe B (Cog5-8). COG functions in the retrograde intra-Golgi trafficking through association with SNARE, Rab and COPI proteins[4,5]. siRNA-directed knock-down of COG subunits induced Golgi ribbon alterations and/or breakdown. Noticeably, specific depletion of Lobe A subunits also led to the accumulation of functional Golgi-derived vesicles that are enriched in glycosyltransferases[6,7].

Quantitative immunoprecipitation experiments with anti-COG IgGs demonstrate that soluble cytoplasmic COG complex is a stable octamer, while 50% of the membrane-bound COG complex partitions into the Lobe A and Lobe B sub-complexes. Depletion of the Golgi SNARE Syntaxin5 augments partitioning of the COG complex into the sub-complexes indicating that Syntaxin5 positively regulates COG complex assembly. Live cell microscopy of HeLa cells stably expressing CFP-Cog6/YFP-Cog3 reveals that Cog6 is associated with vesicles that likely correspond to the intra-Golgi trafficking intermediates. This hints that the transient association of COG sub-complexes is the initial step in intra-Golgi vesicle tethering.

Medial/trans Golgi enzymes remain associated with Golgi membranes in COG complex depleted cells treated with Brefeldin A (BFA). BFA-induced relocalization of cis/medial Golgi enzyme is less dependent on the function of the COG complex, indicating that COG complex operates on the *trans-to-cis* step of the intra-Golgi retrograde trafficking.

Depletion of COG complex lead to the increase in GSII and GNL lectins staining of cell surface, indicating defective terminal protein sialylation and galactosylation. MALDI-TOF analysis of glycoproteins from the COG depleted cells also demonstrates a significant decrease in

the intensity of the complex N-linked sugars. Protein glycosylation defects are similar in cells depleted of either Lobe A or Lobe B, suggesting that both COG subcomplexes regulate the same step in the retrograde intra-Golgi trafficking.

Supported by grants MCB-0645163 and GM083144

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Program/Abstract# 84

Hyperglycemia induces intracellular hyaluronan synthesis and autophagy in dividing rat mesangial cells

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Rat mesangial cells stimulated to divide in hyperglycemic medium up-regulate PKC and synthesize hyaluronan (HA) inside the cells within 15 h during cell division (1). This initiates an autophagic response that facilitates extrusion of an HA matrix that is monocyte-adhesive with concurrent up-regulation of cyclin D3 and C/EBPa, a factor involved in adipogenesis. This mechanism also occurs in trabecular bone marrow of 4 week diabetic (streptozotocin-treated) rats. MicroCT showed loss of trabecular bone mineral in tibia without apparent loss of underlying collagen matrix as would be expected in an osteoclastic response. Demineralized sections showed extensive HA in trabecular bone marrow with embedded autophagic adipocytes and mononuclear monocytes and macrophages. Thus, a chronic inflammatory response appears responsible for the demineralization. This suggests that osteoblastic bone marrow stromal cells can divert to adipogenesis in response to hyperglycemia. This was tested by culturing rat bone marrow stromal cells in osteogenic medium (5.6 mM glucose) compared with hyperglycemic (25.6 mM glucose) osteogenic medium. Cells in the hyperglycemic medium diverted to the autophagic response, produced an extensive monocyte-adhesive HA matrix, and accumulated large amounts of neutral lipids and phospholipids. This pathological adipogenic response provides efficient ways to decrease hyperglycemic glucose (synthesis of HA and lipids), but also creates an HA matrix that can cause chronic inflammatory responses by recruiting monocytes and macrophages, which are necessary to remove the matrix. This adipogenic response likely contributes to many diabetic pathologies. (1)J. Ren, V. Hascall, A. Wang (2009) J. Biol. Chem. In press.

Program/Abstract# 85

Role of SHAP-hyaluronan complex in ovalbumin-induced airway hyperresponsiveness in mice

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Formation of the covalent complex between hyaluronan (HA) and serum-derived hyaluronan-associated proteins (SHAP, derived from the heavy chains of inter-α-trypsin inhibitor) has been found to associate with various inflammatory diseases, such as arthritis and hepatitis. Here we examined the role of SHAP-HA complex in pulmonary inflammation. Airway hyperresponsiveness was induced in mice by ovalbumin (OVA) immunization followed by OVA inhalation for 2 weeks. Measurement of enhanced pause (Penh) value revealed that the early phase response to inhaled OVA was normal, but the late phase response was significantly increased in the SHAP-HA complex-deficient knockout (KO) mice. The KO mice also showed higher response to inhaled methacholine (Mch). ELISA results showed comparable serum level of OVA-specific IgE, but higher serum level of OVA-specific IgG1 in KO mice. Histological analysis of lung showed significant accumulation of HA in the airway after OVA challenge. However, no significant difference was observed between KO and WT mice. The HE staining, Giemsa staining, elastica van Gieson staining and periodic acid-Schiff stain staining also showed comparable results. Analysis of bronchoalveolar lavage fluid (BALF) indicated that KO mice had more macrophages and neutrophils. The levels of major Th1 and Th2 cytokines were comparable, while the levels of soluble tumor necrosis factor receptor-1 (sTNFR1) and interleukin (IL)-12p40 were decreased in KO mice. The results suggest that in murine model of asthma, the SHAP-HA complex participates in a negative feedback system regulating the

pathogenesis of airway hyperresponsiveness and allergic airway inflammation.

Program/Abstract# 86

Diagnostic accuracy of serum glycoprotein levels and fucosylation for the differential diagnosis of liver diseases measured by ELISA

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Background: The level of human serum alpha fetoprotein (AFP) and alpha 1-acid glycoprotein (AGP) are known to be increased as well as alteration of their glycosylation occurs in several pathological conditions. ***Methods***: The aim of the present study is to monitor the changes of AFP and AGP levels and pattern of glycosylation in chronic hepatitis B (HBV-CH), hepatitis B cirrhosis (HBV-LC), hepatitis C cirrhosis (HCV-LC) and alcoholic cirrhosis (ALC) patients' sera along with sex and age–matched healthy individuals. The concentration of AFP and AGP in different patients' group and healthy individuals was measured by ELISA using mAb-AFP and mAb-AGP respectively. Fucosylation levels were determined by ELISA using fucose binding *Aleuria aurantia* lectin. Distribution of sialic acid linkage was tested by two sialic acid specific lectins, *viz*., *Maackia amurensis* agglutinin (MAA) and *Sambucus nigra* agglutinin (SNA) respectively by ELISA. The change in the degree of antennary oligosaccharide chain was also measured by Concanavalin A. ***Results:*** Among patient groups ALC and HCV-LC showed highest level of AFP and AGP respectively which was recorded lowest in HBV-CH. There was also significant change in fucosylation of AFP and AGP in all liver cirrhosis patients except HBV-CH; of them HCV-LC ($p<$ 0.001) was highest. ALC, HBV-CH and HBV-LC have significantly lower MAA / SNA ratio, indicating the predominance of α 2®6 linked sialic acid in AFP. On the contrary, higher MAA/SNA ratio in HCV-LC patients indicated a major change in the sialic acid linkage containing high amount of α 2®3 linked sialic acid. However, no change was noticed in the distribution of sialic acid in AGP. There was no change in antennary oligosaccharide chain of AFP and AGP in HBV-CH patients whereas major change was observed in HBV-LC patients. ***Conclusion:*** This study focussed increased serum AFP and AGP levels, fucosylation as well as predominance of specific sialic linkages among the two glycoproteins which could be useful parameters not only as diagnostic biomarkers but to assess the prognosis of the disease.

Program/Abstract# 87

Identification of new soluble oligosaccharide structures in CDG-I deficient cells

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Protein N-glycosylation is initiated by the dolichol cycle in which the oligosaccharide precursor $Glc_3Man_9GlcNAc_2$-PP-dolichol is assembled in the endoplasmic reticulum (ER). One critical step in the dolichol cycle concerns the availability of Dol-P at the cytosolic face of the ER membrane. In RFT1-deficient patient's cells, the lipid-linked oligosaccharide (LLO) intermediate $Man_5GlcNAc_2$-PP-Dol accumulates at the cytosolic face of the ER membrane. Since Dol-P is a rate-limiting intermediate during protein N-glycosylation, continuous accumulation of $Man_5GlcNAc_2$-PP-Dol would block the dolichol cycle. Hence, we investigated the molecular mechanisms by which accumulating $Man_5GlcNAc_2$-PP-Dol could be catabolized in RFT1-deficient patient's cells. On the basis of metabolic labelling experiments and compared to control human fibroblasts, we identified new soluble oligosaccharide strcuctures and demonstrated that they originated from the accumulating LLO intermediates. In addition, these structures were also detected in other CDG patients' cells accumulating specific LLO intermediates at different cellular locations.

Program/Abstract# 88

Carbohydrate-binding proteins in the nucleocytoplasmic compartment of plant cells

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During the last decade evidence was obtained that plants synthesize carbohydrate-binding proteins upon exposure to stress situations like drought, high salt, hormone treatment, pathogen attack or insect herbivory. In contrast to the 'classical' plant lectins, which are typically found in storage vacuoles or in the extracellular compartment this

new class of lectins is located in the cytoplasm and the nucleus. Based on these observations the concept was developed that lectin-mediated protein-carbohydrate-interactions in the cytoplasm and the nucleus play an important role in the stress physiology of the plant cell. Hitherto, several families of nucleocytoplasmic lectins have been identified. An overview of our current knowledge on the occurrence and distribution of nucleocytoplasmic plant lectins will be given. In addition, the carbohydrate-binding properties of these lectins and potential substrates in the nucleocytoplasmic compartment will be discussed in view of the physiological role of the lectins in the plant cell.

Program/Abstract# 89

Expression of recombinant human Lewis fucosyltransferase III in plant

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Production of recombinant proteins in plants is a major challenge due to lower costs, the lack of mammalian pathogens as the reduction of endotoxin accumulation in plant extracts. But technical and regulatory hurdles remain, including the long initial lead time for production, regulatory uncertainties, and questions about the suitability of plant *N*-glycans in human therapeutics (Andersen and Reilly, 2004); these reasons may explain why no commercial plant derived products are currently available (Pujol *et al*., 2005). *N*-glycosylation is an important break for such biotechnology. In fact, plant *N*-glycosyaltion have complex type *N*-glycans lacking sialic acid, and harboring β(1,2)-xylose which was not found on mammalian complex type *N*-glycan and Fuc which is present but differently linked (Ahn *et al*., 2008). Plant *N*-glycans are fucosylated in α(1,3) to the proximal *N*-acetylglucosamine (GlcNAc) of the chitobiose. In some cases Fuc residue is linked to the *O*-4 GlcNAc at the non-reducing end of the lacto-*N*-biose. hFucTIII is a mammalian fucosyltransferase (FucT) which was able to bind Fuc in α(1,3) and/or α(1,4) on the lacto-*N*-biose producing some Lewis epitopes. If α(1,4)-fucosylation was clearly demonstrated in plant (Fitchette-Laine *et al*. 1997, Lhernould *et al*. 1997, Mélo *et al*. 1997, Wilson *et al*. 2001, Léonard *et al*. 2002), no α(1,3)-linkage on lacto-*N*-biose was demonstrated. To increase the amount and the diversity of lewis determinant product by plants we make tobacco transgenic plant over-expressing *hFUT III*. Fucosyltransferase assays, western blot and mass spectrometry were used to identify, quantify and analyse Le^{a} *N*-glycans. We found that constitutive over-expression of hFucT III activity had no effect on Le^{a} *N*-glycans accumulation. Our results suggest that tobacco recombinant hFucT III acts more as a hydrolase than as a transferase. The question now is why?

Program/Abstract# 90

Role of β-N-acetylhexosaminidases in the formation of paucimannosidic N-glycans in plants

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Plant glycoproteins contain substantial amounts of paucimannosidic N-glycans lacking terminal GlcNAc residues at their non-reducing ends. It has been proposed that this is due to the action of β-hexosaminidases during late stages of N-glycan processing or in the course of N-glycan turnover. Recently we have characterized the three members of family 20 glycoside hydrolases from *Arabidopsis thaliana*. When heterologously expressed in insect cells, these β-hexosaminidases (termed HEXO1-3) could all hydrolyze the synthetic substrates *p*NP-GlcNAc, *p*NP-GalNAc, MU-GlcNAc, MU-GlcNAc-6SO_4 and chitotriose-PA, albeit to a varying extent. With different N-glycan substrates, HEXO1 and HEXO3 displayed a much higher specific activity than HEXO2. To investigate the biological function of the different HEXO proteins in more detail we generated knockout plants lacking HEXO1, HEXO2 or HEXO3 proteins. Analysis of β-N-acetylglucosaminidase activity with synthetic and N-glycan substrates revealed that only HEXO1 and HEXO3 were active *in planta*, whereas no HEXO2-specific activity was found in extracts from leaves or roots. In addition, in leaves and roots from the *hexo1hexo3* double and *hexo1hexo2hexo3* triple knockout line no truncated N-glycans were detectable, which strongly indicates that HEXO1 and HEXO3 are responsible for the formation of paucimannosidic structures in *A. thaliana*. Life-cell imaging and subcellular fractionation showed that HEXO1 is predominately a vacuolar protein. In contrast, HEXO3 is mainly located at the extreme cell periphery in the plasma membrane or cell wall. These results indicate that HEXO1 acts as a "classical" vacuolar hexosaminidase that participates in N-glycan trimming in the vacuole, while HEXO3 might act on secreted glycoproteins.

Program/Abstract# 91

Molecular cloning and characterization of glucosyltransferases from Gerbera hybrida

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The ornamental flower gerbera (*Gerbera hybrida)* has been used as a model to study the molecular basis of flower development in the *Asteraceae*. We report here molecular cloning and characterization of gerbera UDP-glucose: flavonoid glucosyltransferases that can catalyze the glucosylation of a broad range of substrates. The collection of GerEsts was electronically probed with the UDP-glucosyltransferase (UGT) signature sequence to identify putative glucosyltransferase genes. Eighteen expressed sequence tags including three clusters and six singletons were identified using this approach, and full lenght cDNAs were isolated. These were cloned into expression vectors, and recombinant enzymes were expressed in *E. coli*. In a glucosyltransferase assay, four of the fusion proteins produced labelled products when different anthocyanins, flavonoids, hydroxycoumarins and UDP-glucose were used as substrates. One of the recombinant enzymes was expressed in a high level and could be isolated over 95% pure. It showed highest activity for compounds bearing a B-ring ortho-dihydroxylated group.

Program/Abstract# 92

Computational analysis of the strength of water-protein interactions in Concanavalin A

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Discrete conserved water molecules are frequently observed in protein crystal structures, and often mediate carbohydrate-protein interactions. The potential to increase ligand affinity by gaining entropy upon displacement of these waters has been suggested, however, if the waters are highly conserved, it indicates that there may be a significant enthalpic penalty associated with their displacement. The plant lectin concanavalin A (Con A) provides an example of such a system. In all crystal structures of Con A, one key water molecule appears in a similar location in the ligand binding site, regardless of the presence or absence of the carbohydrate ligand, and independent of variations in the carbohydrate sequence or size. Experimental attempts to displace this water with synthetic ligands have thus far failed. Using crystallographic data and computational thermodynamic integration methods, the binding free energy of this water has been determined and found to be significantly higher than that associated with bulk water. In addition, steered molecular dynamics simulations suggest that hydrogen bonds between the ligand and this water are as strong or stronger than those to the protein surface. Crucial insight into the pathway for ligand binding and the thermodynamics of desolvation in Con A are thus provided, which may be pertinent to carbohydrate-based drug design in other systems.

Program/Abstract# 93

Cellulase assays with the region-specifically substituted p-nitrophenyl-β-D-glucopyranoside and cellobioside on the glycon portion

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Functionalized cellulose derivatives have been utilized in many industrial fields as biodegradable materials. Methyl cellulose, for instance, is recognized and hydrolyzed by crude cellulase from *Trichoderma viride* in spite of the non-natural structural modification. However, the enzymatic system and the influence of pendant on the hydrolysis (*i.e.* substituent patterns and the enzymatic recognition) are not still well known. *p*-Nitrophenyl-β-D-glucopyranoside (*p*NP-β-Glc) and *p*-Nitrophenyl-β-D-cellobioside (*p*NP-β-Glc_2) are commonly used for assaying β-glucosidase (BG) and cellobiohydrolase (CBH) being essential for breakdown of cellulose. Our research aims novel synthesis of the chromophoric substrates having a substituent on glycon portion to check the influence on hydrolysis. Since now, 4 kinds of mono-*O*-methylated *p*NP-β-Glc, which were region-specifically substituted at *O-2*, *O-3*, *O-4* and *O-6* positions, and *p*NP-β-Glcs having various ether groups at *O-6* position are synthesized. From their hydrolytic assay by several fungal BG, the 6-OH of glucose was found to be special position where enzyme substrate recognition was relatively loose [1]. In this presentation, we also describe the novel synthesis of region-specifically substituted *p*NP-β-Glc_2 at 6-OH or 6'-OH or both (for example, by trityl and methyl group), which will be subjected to their susceptibility test by CBH like cel6A or cel7A having a known 3D structure with their active site. More work is being done for the synthesis of higher D.P. cello-oligosaccharide [2] with the chromophore for assaying endo-type cellulase.

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Program/Abstract# 94

Complexation of 1,6-Anhydro-maltooligosaccharides and their Glycoconjugates

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The 1,6-anhydro-β-D-hexopyranose possesses the steric rigidity adapting the $^1C_4$ conformation in the crystalline state, as well as in solution, whereas the corresponding D-hexopyranose and its glycoside generally occur in the $^4C_1$ conformation. Such a characteristic feature determines the chemical and physical properties even in the 1,6-anhydr-β-maltooligosaccharide having 1,6-anhydro-β-D-hexopyranose moiety. The abilities of 1,6-anhydro sugars to form complex with alkali metal ion have been suggested earlier, since the electrophoretic studies provided information that the behavior of 1,6-anhydro-β-D-glucopyranose was an exception in carbohydrates. Recently, complex formation of 1,6-anhydro-maltooligosaccharides with alkali metal ions has been characterized by spectroscopic analysis. According to 1,6-anhydro-disaccharide, X-ray crystallographic analyses were accomplished for the complex of 1,6-anhydro-β-maltose with sodium and that with potassium, whereas the complexation with rubidium was characterized using ^{1}H DOSY, ^{87}Rb NMR spectroscopy and electrospray ionization mass spectrometry. The complex formation of 1,6-anhydro-β-maltotriose with potassium ion was also characterized using ^{1}H and ^{39}K NMR spectroscopy. The complex formation selectivity of 1,6-anhydro-β-maltooligosaccharide with each alkali metal ion has been found to alter with respect to the oligosaccharide length.

Program/Abstract# 95

Investigating the roles of Mucin-type O-glycosylation during eukaryotic development

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Mucin-type O-glycosylation represents a major form of post-translational modification that is conserved across most eukaryotic species. This type of glycosylation is initiated by a family of enzymes (ppGalNAcTs in mammals and PGANTs in Drosophila) that catalyzes the transfer of GalNAc to the hydroxyl group of either serine or threonine in protein substrates. There are 9 genes encoding functional PGANTS Drosophila and as many as 18-20 in mammals. In an effort to define the developmental roles of O-glycosylation, we have employed Drosophila as our model system to circumvent the functional redundancy inherent in this enzyme family. Previous work from our group demonstrated that these genes are expressed in distinct spatial and temporal patterns throughout Drosophila development, suggesting unique functions for the enzymes they encode. Loss-of-function mutations in one family member, pgant35A, resulted in death during embryogenesis and irregularities in epithelial tube formation, indicating that this protein is essential for viability. Mutations in another family member, pgant3, disrupted proper cell adhesion during development. Ongoing studies examining conventional mutations as well as reductions in pgant gene expression using RNA interference continue to reveal additional roles for members of this family. Our results suggest that multiple members of the PGANT enzyme family serve unique and essential functions during Drosophila development, providing insight into the role of this highly conserved protein modification during mammalian development.

Program/Abstract# 96

Functional roles of sperm Hyaluronidases, HYAL5 and SPAM1, in mouse fertilization

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Although the sperm entry into the oocyte-cumulus complex and subsequent sperm penetration through the cumulus matrix to reach the oocyte zona pellucida are essential for mammalian fertilization, the molecular mechanism still remains controversial. We have previously shown that mouse sperm lacking SPAM1 are capable of penetrating the cumulus matrix despite a delayed dispersal of cumulus cells. We also identified another sperm hyaluronidase HYAL5 as a candidate enzyme involved in sperm penetration through the cumulus. In this study, we have produced HYAL5-deficient mice to uncover the functional roles of HYAL5 and SPAM1 in fertilization. The HYAL5-deficient mice were fully fertile and yielded normal litter sizes. *In vitro* fertilization assays demonstrated that HYAL5-deficient epididymal sperm is functionally normal. We thus conclude that HYAL5 may be dispensable for fertilization. Comparative analysis among wild-type, HYAL5-deficient, and SPAM1-deficient epididymal sperm revealed that only SPAM1 is involved in sperm penetration through the cumulus matrix. Notably, the loss of SPAM1 resulted in a remarkably increased accumulation of sperm on the surface or outer edge of the cumulus. These data suggest that SPAM1 may function in the sperm entry into the cumulus and the sperm penetration through the cumulus matrix.

Program/Abstract# 97

MicroRNAs regulate Heparan sulfate proteoglycan's function

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Accumulating evidences indicate that heparan sulfate proteoglycans (HSPGs) are essential for cell growth and development. Most miRNAs through interaction with the 3′ UTR posttranscriptionally suppress the targeting mRNA expression. The common standard is experimental demonstration that a luciferase reporter fused to the 3-UTR of the predicted target is repressed by overexpression of the miRNA. Given the wide impact of miRNAs on gene expression, it is not surprising that a number of miRNAs have been implicated in HSPGs biology. Here, we demonstrate for the first time a functional link between HSPGs, their well documented biosynthetic and degraded enzymes acting sequentially, and miRNA expression. We used luciferase reporter constructs containing the HSPGs and the enzymes 3′ UTR with the putative binding site downstream of the reporter coding region. By cotransfection of the miRNAs to screen the HSPGs and the enzymes' target miRNA. Our data indicate that certain miRNA repressed HSPGs and the enzymes, such as mir-125a, b inhibiting glypican-4, mir-330 and mir-200a repressing sulf2, mir-100,mir-99a, mir-194, mir-218, and mir-224 target 3-OST-2 etc. Interestingly, mir-125a interrupts ERK phosphorylation implicated in glypican function. Furthermore, microarray-based miRNA expression stimulated with exogenous polysaccharide profiles reveal that a specific spectrum of miRNAs are regulated (including mir-518b, mir-200a, mir-125a, b, mir-330 etc). Our study provides the first clues that miRNA may regulate HSPG homeostasis in the process of cell growth and development.

Program/Abstract# 98

Site directed processing: role of amino acid sequences and glycosylation of acceptor glycopeptides in the assembly of extended mucin type O-glycan core 2

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The assembly of Ser/Thr-linked O-glycans of mucins with core 2 structures is initiated by polypeptide GalNAc-transferase (ppGalNAc-T), followed by the action of core 1 β3-Gal-transferase (C1GalT) and core 2 β 6-GlcNAc-transferase (C2GnT). β4-Gal-transferase (β4GalT) extends core 2 and forms the backbone structure for biologically important epitopes. In chronic diseases, mucin expression as well as O-glycan structures are often abnormal. The goal of this work is to determine if the activity and specificity of these enzymes is directed by the sequences and glycosylation of substrates. We studied the specificities of these four enzymes using as acceptor substrates synthetic mucin-derived peptides and glycopeptides, substituted with GalNAc or O-glycan core structures 1, 2, 3, 4 and 6. Specific Thr residues were found to be preferred sites for the addition of GalNAc, and Pro in the +3 position was found to especially enhance primary glycosylation. An inverse relationship was found between the size of adjacent glycans and the rate of GalNAc addition. All four enzymes could distinguish between substrates having different amino acid sequences and O-glycosylated sites. A short glycopeptide was identified as a highly efficient C2GnT substrate. The activities of all four enzymes assembling the extended core 2 structure are affected by the amino acid sequence and presence of carbohydrates on nearby residues in acceptor glycopeptides. Knowledge of site directed processing enhances our understanding of the control of O-glycosylation in normal cells and in disease. The work was funded by the Canadian Cystic Fibrosis Foundation.

Program/Abstract# 99

Enhancement / suppression of the activity of bovine testicular and human cancer cell hyaluronidases by formation of hyaluronan-protein complexes

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Hyaluronan (HA) is a high-molar-mass glycoaminoglycan widely distributed in the extracellular matrix (ECM) of vertebrate tissues. Its physiological functions are related to its unique physicochemical properties and also to its ability to interact with various proteins including membrane receptors like CD44. HA is involved in many biological processes such as cellular proliferation, mobility and differentiation. However, the functions of HA depend on chain size. Hyaluronidases (HAase), by catalysing HA hydrolysis, play an important role in the control of the size and concentration of HA chains. Levels of HA and HAase significantly increase in several tumors where they play a role in cancer invasion. When studying the effect of HA and HAase concentrations on the kinetics of HA enzymatic hydrolysis, using testicular HAase as a model enzyme, we

observed an atypical behaviour. We showed that this behaviour could be explained by the ability of HA to form electrostatic complexes with HAase which thus becomes unable to catalyse HA hydrolysis. For that purpose, we used albumin as a non-catalytic protein able to compete with HAase for the formation of electrostatic complexes with HA, allowing HAase to be free and catalytically active. In fact, albumin can either enhance or suppress HAase activity depending on its content with respect to the HA and HAase concentrations. *In vitro* experiments were also performed with HAase from lung carcinoma cells and showed the same enhancement / suppression of HAase activity by protein. Interestingly, these results fit well with the observations made on HA and protein contents in human tumors. It also explains the regression of tumors treated with proteins. All of this suggests that the HA/protein/HAase balance in the ECM can modulate invasion.

Program/Abstract# 100

Characterization of glucosylceramides in Scedosporium apiospermum and their involvement in fungal differentiation

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Scedosporium apiospermum (teleomorph *Pseudallescheria boydii*) is a saprophytic filamentous fungus recognized as a potent agent of a wide variety of infections in immunocompromised, as well as in immunocompetent patients [1].

Fungal cerebrosides (CMHs) have conserved structures and have been related with sorting of molecules to the cell surface, cell differentiation and growth, and pathogenicity [2].

Ceramide monohexosides (CMHs) were purified from lipid extracts of *S.apiospermum* mycelium by successive chromatographic steps [3] showing that, as for many other species, it synthesizes glucosylceramides (CMHs) as major neutral glyosphingolipids. They were analyzed by HPTLC, GC-MS, ESI-MS and NMR spectroscopy. A Glc-Cer enriched fraction, on+ion ESI-MS, gave a cluster of singly-charged ions with *m/z* from 700 to 800 with a profile similar to that described for other fungal cerebrosides [2]. A major species with *m/z* 735 [Li^+] corresponded to *N*-2`-hydroxyhexadecanoyl-9-methyl-4,8-sphingadienine. Monoclonal antibodies against CMHs were used in differentiation assays of *S. apiospermum*, demonstrating that binding of anti-CMH antibodies to the fungal surface results in inhibition of the morphological transition of conidia to mycelia. We showed that fungal CMHs are involved in differentiation, and consequently play a role in infectivity of fungal cells. Determination of structural and functional aspects of these fungal glycoconjugates could contribute to the design of new agents capable of inhibiting fungal growth and differentiation of pathogens.

Supported by: CNPq, FAPERJ, CAPES, PRONEX

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[3] da Silva A.F. *et al*. 2004. FEBS Letters 561: 137–143

Program/Abstract# 101

Novel UDP-Gal derivative interferes with active site closure and inhibits transfer in human blood group glycosyltransferases

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The ABO(H) blood group A and B glycosyltransferases catalyse the final step in the synthesis of the A and B antigens. The N-acetylgalactosaminyltransferase (GTA) converts the H-antigen acceptor to the A antigen by transferring the GalNAc moiety from the UDP-GalNAc donor whereas the galactosyltransferase (GTB) uses UDP-Gal donor to make the B antigen. GTA and GTB differ by only four amino acid residues (R/G176, G/S235, L/M266, G/A268) and the specificity can be modified by replacement of these residues. One such example is the cis-AB mutant (GTA-L266G/G268A) capable of transferring both Gal and GalNAc to the H-antigen with equal efficiency. We have developed a novel derivative of UDP-Gal with an aromatic or heteroaromatic substituent in position 5 of the uracil base as chemical tools for the investigation of glycosyltransferases and other UDP-Gal-dependent glycoprocessing enzymes. The UDP-Gal derivative is prepared from uridine in five synthetic steps and it binds very tightly to the active site of the several tested glycosyltransferases with K_i values in the in the low uM range. Furthermore, we have solved the high-resolution crystal structure of the cis-AB mutant with the full UDP-Gal derivative bound to active site. The structure shows that the derivative binds in an identical manner to the UDP-Gal donor. A flexible active site loop and the C-terminus normally goes from an open to a closed conformation upon donor binding, where Trp181 in the active site loop and Arg352 in the C-terminus form a stacking interaction above the uracil moiety. This interaction is sterically prevented by

the substituent on the UDP-Gal derivative and leads to an almost complete inhibition of Gal transfer.

Program/Abstract# 102

The sugar paved road towards improved stem cell therapy

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Stem cells hold an enormous therapeutic potential in regenerative medicine. However, before stem cells can be used in the clinical practice, there is a need for methods to characterize them, to distinguish them from other cells, and to control variation between preparations. Despite the rapid expansion of the stem cell field, standardized safe practices for isolating and culturing human stem cells for clinical use have not been established yet. In addition, targeting of therapeutic cells to the relevant tissues is in many cases ineffective. Stem cell glycomics provides an ideal platform to solve these issues, since glycosylation is known to be cell type specific, change during development and differentiation, and play important roles in cell adhesion and migration. To get an overview of stem cell glycobiology, we have studied both pluripotent embryonic stem cells and multipotent adult stem cells (mesenchymal and hematopoietic). A panel of complementary methods including mass spectrometry, various immunochemical methods and transcript profiling was used to characterize the glycomes of stem cells and differentiated cells. Characteristic glycosylation features that change upon differentiation were revealed for the different stem cell types. We are using the information on stem cell glycomes to develop methods to evaluate the differentiation stage of stem cells, detect xenocontamination, enrich stem cell populations, improve culture conditions, and manipulate stem cell surfaces in order to achieve optimized biodistribution upon therapeutic administration. In particular, we have developed a defined lectin based matrix for the culture of embryonic stem cells and induced pluripotent (iPS) cells, to replace complex growth supports containing animal derived material. The impact of glycosylation on the biodistribution of stem cells has been demonstrated by showing that differentially glycosylated mesenchymal stem cell types display different migratory behaviour *in vivo*. Understanding the glycosylation of stem cells will help to develop more effective and safe therapies.

Program/Abstract# 103

Role of polysialic acid and NCAM in postnatal neurogenesis

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Understanding the mechanisms that regulate neurogenesis is a prerequisite for the potential use of neuronal precursors in brain repair approaches. One important candidate regulator of postnatal neurogenesis is polysialic acid (polySia), a posttranslational modification of the neural cell adhesion molecule NCAM. Animals that are devoid of polySia but have normal expression levels of NCAM exhibit a severe phenotype with specific brain wiring defects, progressive hydrocephalus, postnatal growth retardation, and precocious death (Weinhold *et al*., JBC 280: 42971). These defects were rescued by additional deletion of NCAM, demonstrating that they originate from a gain of NCAM functions due to polySia-deficiency. In the present study, we investigated the role of polySia and NCAM in differentiation of precursors isolated from the subventricular zone of early postnatal mice, *i.e.* from the major germinal niche persisting into the adult brain. We demonstrate that loss of polysialic acid promotes cell survival and neuronal differentiation. Neurite induction and maturation into a calretinin-positive phenotype is enhanced by removing polySia and by exposure to trans-interacting NCAM. Notably, precursors isolated from NCAM-deficient mice responded to NCAM cues in exactly the same way as wild-type cultures indicating the involvement of heterophilic NCAM binding. NCAM interactions also account for the higher degree of differentiation observed in precursors isolated from polysialic acid-negative mice. Revealing that expression of polySia specifically controls NCAM trans-interactions provides new options to direct neurogenesis from subventricular zone-derived precursors.

Program/Abstract# 104

Linking glycan expression to pathway dynamics during stem cell differentiation

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Glycosylation changes accompany cellular differentiation in developing tissues. However, assigning glycan expression changes to specific cellular identities is not possible using standard tissue glycan profiling because maturing tissues are composed of multiple cell types. However, embryonic stem cells recapitulate many of the cellular changes associated with tissue maturation as they undergo differentiation in vitro, offering a means to investigate glycomic dynamics independent of the complicating cellular heterogeneity of tissue extracts. We have characterized the changes in N-linked, O-linked, and glycosphingolipid

glycans resulting from mouse embryonic stem cell (ES) differentiation, induced by retinoic acid to become extra-embryonic endoderm (ExE) or by removal of LIF to become embryoid bodies (EB). Analysis of transcripts (qRT-PCR) related to glycan synthesis and processing has probed for correlations between gene expression and glycan production. Mouse ES cell differentiation is characterized by decreasing high-Man and increasing complex N-linked glycans. Increases in the transcripts for enzymes involved in specific terminal modifications of N-linked glycans (sialylation and GalaGal) are concordant with increases in their expected products. Terminal O-linked sialylation is enhanced during differentiation and the distribution of O-linked cores also changes. Glycosphingolipid profiles are characterized by increasing levels of gangliosides, especially for GM3 and GD3, although GD1 decreases in EB and ExE. For all glycan classes, some changes in glycans and transcripts correlate as expected while others do not, consistent with the expectation that the regulation of complex glycosylation pathways may not always rely on the expression of a single enzyme.

Program/Abstract# 105

Quantitative glycomics during cellular differentiation

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It has known the glycosylation plays an important role in the biological processes, such as cell adhesion, proliferation, and differentiation. Although genomic and proteomic approach during cell differentiation have been intensively studied to discriminate cellular stages, glycomics have been laggard compared to them due to their complex structures and hardness of quantification. Recently we have developed "glycoblotting" technique, a high-throughput and quantitative method for comprehensive glycomics, which enables to enrich and quantify glycans from crude biological materials.>P19C6 and P19CL6 cells, the subclones of P19 mouse embryonic teratocarcinoma cell line, have served as the well-established models for studying neuronal and cardiac muscle differentiation process after retinoic acid and DMSO inducement, respectively. In this study we have analyzed *N*-glycome of P19C6 cells with or without inducement into neuronal cells, as well to P19CL6 cells into cardiomyocytes. We focused on the changes in *N*-glycomes that have been known to contribute to cell differentiation. Total cell glycoproteins were obtained from the acetone-precipitated fraction of cell lysates followed by reductive alkylation. In order to profile total cellular *N*-glycans, they were released from the glycoproteins by digestion with PNGase F after trypsin digestion. They were enriched and purified by glycoblotting, and then applied to MALDI-TOF MS and MS/MS analysis. The results suggested that glycomics utilized glycoblotting can be useful to judge cellular differentiation stage with quantitative information and high sensitivity, which cannot be provided only by proteomics or genomics. We will also show the time-dependent change of *N*-glycome in mouse ES and iPS cells under differentiation.

Program/Abstract# 106

Expression and possible functions of gangliosides in neural stem cells

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Neural stem cells (NSCs) are undifferentiated neural cells endowed with the proliferative potential, the capacity for self-renewal and the multipotency to differentiate into neurons and glial cells. In a series of the studies, we have investigated the expression pattern and the possible functions of gangliosides, sialic acid-containing glycosphingolipids, in the NSCs. In primary mouse neuroepithelial cells (NECs) which are rich in NSCs, b-series gangliosides such as GD3, GT1b and GQ1b were found to be abundantly expressed. In the NECs treated with PDMP, an inhibitor of glycosphingolipid synthesis, the ganglioside expression and cytokine-induced ERK (MAPK) activation and proliferation were repressed. Disruption of glycosphingolipid-enriched microdomains, lipid rafts, by methyl-beta-cyclodextrin also repressed the cytokine-induced ERK activation. Based on these results, we proposed the possibility that gangliosides are involved in the fate regulation of NSCs by regulating the signal transduction via lipid rafts. On the other hand, no significant difference in the viability, proliferation rate, differentiation characteristics and cytokine-mediated signaling pathways was found in NECs prepared from GD3-synthase knockout mice. These results indicate that GD3 is not essential for the fate regulation of NECs, at least in GD3-synthase knockout mice, and the functions of GD3 in NECs may be compensated to some extent by other glycosphingolipids such as a-series gangliosides.

Program/Abstract# 107

The 3′-phosphoadenosine 5′-phosphosulfate transporters, PAPST1 and PAPST2, are important for the maintenance and the differentiation of mouse embryonic stem cells

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Sulfation is an essential modification of many carbohydrates and proteins, and is necessary for normal growth and development. Sulfation of macromolecules requires the translocation of a high-energy form of nucleotide sulfate, *i.e.*, 3'-phosphoadenosine 5'-phosphosulfate (PAPS), from the cytosol into the Golgi apparatus. Recently, we have identified and characterized two homologues of PAPST transporters, PAPST1 and PAPST2 in both human and *Drosophila*. Mutation and RNA interference (RNAi) of each *Drosophila PAPST* have induced the lethality and the morphological defects to show the importance of PAPST mediated sulfation of carbohydrates and proteins during development. In this study, we investigated the contribution of PAPST mediated sulfation to the maintenance and the differentiation of mouse embryonic stem (ES) cells.
At first, we determined the PAPS transport activity of mouse PAPST1 and PAPST2 proteins by using a yeast expression system. Both proteins showed similar PAPS transport activities with an apparent Km value of 1.5 mM. RNAi of each *PAPST* resulted in the reduction of sulfation. Each *PAPST* knock down ES cells exhibited a flattened and differentiated morphology and the reduced expression of stemness genes. It demonstrated that PAPST mediated sulfation is important for the self-renewal of ES cells. RNAi of each *PAPST* also showed the decreased cell proliferation. During the differentiation of *PAPST*-knockdown ES cells, they exhibited abnormal phenotype. We will discuss the role of PAPST mediated sulfation in mouse ES cells including signaling pathways.

Program/Abstract# 108

Cryptic glycan epitopes in necrotic mesenchymal stem cells

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Mesenchymal stem cells (MSC) are adult multipotent progenitor cells that can be isolated from various tissues like bone marrow, adipose tissue and cord blood. MSCs are able to differentiate into osteoblasts, chondroblasts and adipocytes and they hold an enormous potential for regenerative medicine. As glycans cover the entire cell surface as the glycocalyx, they play an important roles in cell adhesion and signalling events. Glycosylation is known to be cell type specific and can be used to characterize cells and to control variation between stem cell lines. We have previously shown that glycosylation changes during stem cell differentiation and others have described glycosylation changes in mammalian cells during apoptosis and necrotic death. Here we show specific glycan epitopes detected in mesenchymal stem cells during necrosis and propose that proteins binding to these glycan epitopes can be used in evaluating the condition of cells. In this study, bone marrow and cord blood MSCs were screened with various glycan binding antibodies using flow cytometry. After cell detachment with EDTA, a small subpopulation of cells was found to be positive for staining with a certain set of glycan binders. These cells were later shown to be necrotic and not apoptotic. nor the detachment of cells with trypsin. The specificities of these glycan binders have now been determined by the Consortium for Functional Glycomics and these antibodies, originally described to be specific for a number of different epitopes, were found to share binding specificity towards a defined set of glycan epitopes.

Program/Abstract# 109

Glycosphingolipid storage diseases and tuberculosis: the unexpected connection

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Niemann-Pick disease type C (NPC) is a lysosomal storage disorder. Defects in two independent genes can cause this disease (NPC1 and NPC2) and the proteins they encode are believed to act in a common cellular pathway. The cellular features of NPC disease include the accumulation of several classes of lipids (cholesterol, sphingomyelin, spingosine and multiple glycosphingolipids) and a block in late endosome-lysosome fusion that results from a unique acidic store calcium defect that we recently identified (1). The intracellular pathogen, *Mycobacterium tuberculosis*, survives within macrophages by inhibiting host cell phagosome-lysosome fusion. Infected cells develop a characteristic cholesterol laden foamy cell phenotype that superficially resembles NPC, raising the possibility that TB and NPC

share convergent mechanistic features to their cellular pathology. We have found that cells infected with *Mycobacterium bovis* (BCG) accumulate all the lipids stored in NPC disease and have reduced levels of lysosomal calcium. The lysosomal calcium defect leads to a failure in phago-lysosome fusion and this finding may explain how the mycobacteria escape clearance. The same NPC cellular phenotypes are induced when mycobacterial lipids are applied to healthy cells, suggesting that these lipid(s) are inhibiting the NPC pathway. Therapies that correct NPC cells promote the clearance of mycobateria. The imino-sugar drug miglustat (*N*-butyldeoxynojirimycin) an inhibitor of glycosphingolipid biosynthesis,promotes phago-lysosome fusion, thereby facilitating the clearance of the mycobacterium. The clinical relevance of these findings to potential TB therapy will be discussed.

1. Lloyd-Evans et al, 2008, Nature Medicine, 14, 1247–1255.

Program/Abstract# 110

Myelin-associated glycoprotein (Siglec 4) protects Axons from acute toxicity via a ganglioside-dependent mechanism

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Gangliosides – sialylated glycosphingolipids – are the major glycans of nerve cells and their axons. The same four ganglioside species, GM1, GD1a, GD1b and GT1b, are common to all mammals, constituting ~95% of the total gangliosides in the brains of humans and rodents alike. Among the functions of gangliosides, GD1a and GT1b are receptors for a nervous system lectin, myelin-associated glycoprotein (MAG). MAG, which resides on the innermost wrap of myelin, contributes to proper axon architecture and long-term axon stability. Mutant mice lacking MAG, as well as mice lacking MAG-binding gangliosides, have impaired axon-myelin structural associations, and progressive axon degeneration evident over a period of months. Recently, MAG was also found to protect axons from acute toxic insults. Dorsal root ganglion neurons cultured on myelin-adsorbed surfaces were resistant to acute degeneration induced by addition of vincristine, a cancer chemotherapeutic agent with neuropathic side effects. Myelin-mediated protection was reversed by anti-MAG antibody and was reversed when cells were cultured on extracts from *Mag*-null myelin, confirming the protective role of MAG. Treatment of neurons with sialidase, an enzyme that cleaves terminal sialic acids required for MAG binding, reversed MAG's protective effect, as did treatment with P4, an inhibitor of glycosphingolipid biosynthesis. We conclude that MAG protects neurons from acute toxic insults via a ganglioside-mediated signaling pathway. Understanding MAG-mediated protection may provide opportunities to reduce axonal damage and loss. Supp. by NIH grant R37NS037096.

Program/Abstract# 111

UDP-sugar precursors infuence hyaluronan synthesis by substrate availability and HAS transcription in keratinocytes

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Hyaluronan controls important pathobiological processes like inflamation and cancers. Its synthesis is stimulated by growth factors through transcription of *HAS* genes. However, less is known about the influence of other than growth factors, or postranscriptional effectors in general. Reduction of UDP-N-Acetyl-hexosamines (UDP-HexNAc) and UDP-glucuronic acid (UDP-GlcUA) by mannose and 4-methylumbelliferone, respectively, inhibited hyaluronan synthesis, due to substrate shortage, while increasing cellular UDP-HexNAc by glucosamine increased hyaluronan synthesis. UDP-HexNAc is a key metabolite in hexosamine synthesis pathway, and a suggested sensor of glucose supply, important in obesity, insulin resistance and type 2 diabetes. We found that UDP-HexNAc content per cell peaked (~doubled) at 6 h after change of fresh medium, but was little affected by raising glucose from 5.5 to 25 mM or decreasing serum from 10 to 0.5%. Interestingly, manipulation of UDP-HexNAc contents by mannose and glucosamine was associated with reciprocal changes in *HAS2* mRNA, as if compensating at the transctiptional level the scarcity and oversupply of UDP-HexNAc. Exploration of the signals mediating the influence of mannose and glucosamine revealed a correlation between STAT transcription factors and *HAS* expression. Chromatin immunoprecipitation confirmed this association and also showed the involvement of the transcription factors YY1, SP1, and the cofactors CBP, NcoR, SMRT and PCAF in the response to mannose and glucosamine. It is concluded that the cellular content of UDP-HexNAc not only influences the rate of the enzymatic reaction producing hyaluronan, but also the transcriptional activity of *HAS* genes, the latter through signaling pathways currently under closer examination.

Program/Abstract# 112

Targeting an antimicrobial effector function in insect immunity as a pest control strategy

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Insect pests such as termites cause damages to crops and man-made structures estimated at over $30 billion per year, imposing a global challenge for the human economy. Here we report that termite GNBP-2 (belonging to the family of gram-negative bacteria binding proteins GNBPs) shows β(1,3)-glucanase effector activity previously unknown in animal immunity and is a pleiotropic pattern recognition receptor and an antimicrobial effector protein. By means of rational design, we present an inexpensive, nontoxic small molecule glycomimetic that blocks tGNBP-2, thus exposing termites in vivo to accelerated infection and death from specific and opportunistic pathogens. Such a molecule, introduced into building materials and agricultural methods, could protect valuable assets from insect pests.

Program/Abstract# 113

The motility of ovarian carcinoma cells is regulated through a Glycosphingolipid/CAveolin-1 signaling complex

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In this paper, we describe the effects of the expression of GM3 synthase at high levels in human ovarian carcinoma cells. Overexpression of GM3 synthase in A2780 cells results in elevated ganglioside levels, reduced *in vitro* cell motility and enhanced caveolin-1 expression. In these cells, caveolin-1 directly interacts with GM3 ganglioside and is associated with sphingolipids, integrin receptor subunits, p130Cas and the non-receptor tyrosine kinase c-Src forming a non-caveolar signaling complex, insoluble in both Triton X-100 and Brij 98 and not containing tetraspanins. The motility of low GM3 synthase expressing cells is reduced in the presence of exogenous gangliosides and of a Src inhibitor; on the other hand, higher levels of the inactive form of c-Src are associated with the ganglioside- and caveolin-rich detergent insoluble fraction in high GM3 synthase expressing cells.

These data suggest a novel role for gangliosides in regulating tumor cell motility, by affecting the organization of a signaling complex organized by caveolin-1, responsible for Src inactivation downstream to integrin receptors, and imply that GM3 synthase is a key target for the regulation of cell motility in human ovarian carcinoma.

Program/Abstract# 114

Segregation of mucin core 2 enzymes, C2GnT-1(L) and C2GnT-2(M), in the Golgi apparatus

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Cell-cell interaction is a major function of mucin-type glycans in membrane glycoproteins while protection of mucus secretory epithelium is the primary function of the glycans in gel-forming secreted mucins. Both functions are controlled by the β6GlcNAc branch structures, *i.e.* core 2 in membrane glycoproteins, and core 2, core 4, and I antigen in secreted mucins. C2GnT-1 is ubiquitously expressed and involved in the synthesis of core 2 of membrane-bound mucin glycans. C2GnT-2 is expressed in mucus-secretory tissues and involved in the synthesis of all three mucin branch structures. C2GnT-1 is a cis-medial Golgi protein, but the Golgi localization of C2GnT-2 is not known. The goal of the study is to determine if C2GnT-2 is co-localized with C2GnT-1 in the Golgi. H292 cells cultured on a glass coverslip were treated with rabbit anti-C2GnT-1 and goat anti-C2GnT-2 antibodies, respectively, and then with DyLight 594 (Red)-labeled donkey anti-rabbit and DyLight 488 (Green)-labeled donkey anti-goat antibodies. Serial Z-sections of green and red images were collected separately under a Laser Scanning Microscope. Calculated 3-D projection images of each fluorescence color were obtained and overlayed to assess co-localization. The result showed that red and green fluorescence colors were largely segregated, suggesting that these two isozymes were localized to different parts of the Golgi apparatus. The result will be further confirmed by Immuno-Gold electron microscopy aided by other known Golgi markers. The surprising finding suggests that secreted mucins utilize a unique path

containing C2GnT-2 for travel through the Golgi apparatus. However, other glycoproteins take a different Golgi path, which contains C2GnT-1. This hypothesis is currently under investigation.

Program/Abstract# 115

Cholesterol depletion suppresses hyaluronan synthesis by downregulating hyaluronan synthase 2 associated with inhibition of Akt and activation of STAT5

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Hyaluronan synthases (HAS) are integral plasma membrane proteins that assemble megadalton polysaccharide hyaluronan (HA) from UDP-N-acetylglucosamine and UDP-glucuronic precursors. In bacterial cells, HAS activity is dependent on the lipid microenvironment and in mammalian cells, HAS is partly localized in cholesterol-rich lipid rafts. Cholesterol depletion suppresses HA secretion in smooth muscle cells, and the effect can be reversed by addition of exogenous cholesterol. Furthermore, aortic smooth muscle cells from hyperlipidemic rabbits and skin fibroblasts from hypercholesterolemic patients secrete 2-4 fold more HA than corresponding controls. We studied the effect of cholesterol depletion by methyl-β-cyclodextrin (MBCD) on HA synthesis in MCF-7 human breast cancer cells. We found that cholesterol depletion suppresses HA synthesis but does not decrease the molecular mass of newly produced HA. Interestingly, addition of exogenous cholesterol only partly restores HA production suggesting other factors than cholesterol content of plasma membrane regulate HAS activity and HA synthesis. MBCD does not decrease levels of the UDP-glucuronic acid and UDP-N-acetylglucosamine but specifically downregulates the mRNA level of HAS2 without affecting other HASes. To find out the signaling pathways that regulate HAS2 expression we screened the activation of signaling proteins after MBCD treatment. Our results indicate that MBCD inhibits phosphorylation of Akt and its downstream target p70S6 kinase while phosphorylation of signal transducer and activator of transcription 5a/b (STAT5a/b) is increased. Experiments exploring the possible binding of pSTAT5 to HAS2 promotor in response to MBCD and signaling proteins upstream and downstream of Akt affected by MBCD are in process.

Program/Abstract# 116

Modulation of biological functions with synthetic carbohydrate-based macromolecules

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We have employed well-defined complex synthetic oligosaccharides and glycoconjugates to probe biological processes and use the resulting information to devise novel therapeutic strategies. For example, we have designed, chemical synthesized and immunologically evaluated a number of fully synthetic vaccine candidates to establish strategies to overcome the poor immunogenicity of tumor-associated carbohydrates and glycopeptides. It has been found that a three-component vaccine composed of a Toll Like Receptor agonist, a promiscuous peptide T-helper epitope, and a tumor-associated glycopeptide, can elicit potent anti-tumor immune responses in mice. A similar strategy has been employed to generate *O*-GlcNAc-specific IgG MAbs having a broad spectrum of binding targets. Large-scale shotgun proteomics led to the identification of 254 mammalian *O*-GlcNAc modified proteins, including a large number of novel glycoproteins. The data imply a role of *O*-GlcNAc in transcriptional/translational regulation, signal transduction, the ubiquitin pathway, anterograde trafficking of intracellular vesicles and post-translational modification. To identify new tumor associated carbohydrate antigens, 4-dibenzocyclooctynol derivatives have been developed as novel click reagents for the isolation and visualization of oligosaccharides of living cells that are metabolically labeled with azido containing monosaccharides. The new reagent has been used for the development of a quantitative glycoproteomics approach.

Program/Abstract# 117

Functional characterization of *Yarrowia lipolytica* homologues of *Saccharomyces cerevisiae MNN4* and *MNN6* genes in mannosylphosphorylation of *N*- and *O*-linked oligosaccharides

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Mannosylphosphorylation is known as a major oligosaccharide modification in the conventional yeast *Saccharomyces*

cerevisiae. Two genes of *S. cerevisiae*, *ScMNN4* and *ScMNN6*, are involved in the mannosylphosphate transfer reaction processed in the Golgi. We previously reported the presence of mannosylphosphate residues in the *N*-glycans of *Yarrowia lipolytica*, a non-conventional yeast species. By homology search, a single and four ORFs showing significant homology to *ScMNN4* and *ScMNN6,* respectively, were identified. Single or double mutant strains of each *Y. lipolytica* homologue were constructed and analyzed for their function in mannosylphosphorylation of *N*- and *O*-glycans. It was observed that the single disruption of *Y. lipolica MNN4* homologue (named *YlMPO1*)only was enough to generate a complete disappearance of acidic sugar moieties both in *N*- and *O*-glycans. Furthermore, the *Ylmpo1Δ* strain showed the decrease of blue-color intensity in the alcian blue staining, suggesting the loss of cell wall mannosylphosphates by the inactivation of *YlMPO1*. In contrast, the other *Y. lipolytica* mutant strains of *MNN6* homologues synthesized truncated forms of *O*-mannosyl glycans without any apparent defects in *N*-glycan biosynthesis and in mannosylphosphate addition. This implies that the functions of the *Y. lipolyticaMNN6* homologues are associated with the elongation of the *O*-glycans by addition of α-1,2-mannose residues, not with mannosylphosphorylation. This is consistent with that they exhibit relatively higher homologies to the members of the *ScKTR* family than to *ScMNN6*. Altogether, our results strongly suggest that *YlMPO1* plays a key role in mannosylphophorylation of both *N*- and *O*-glycans without involvement of a *MNN6* homologue in *Y. lipolytica*, differently from in *S. cerevisiae.*

Program/Abstract# 118

Glycosylation prevents moisture-induced instabilities of alpha-chymotrypsin in the solid state

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The employment of proteins as biotherapeutic agents has gained increased interest due to their chemical selectivity and catalytic efficiency when compared to small molecules. Solid protein pharmaceuticals often suffer from instability problems due to moisture adsorption thus hampering their successful therapeutic application. One frequently formulated hypothesis is that partial protein hydration from moisture adsorption increases protein structural motions leading to protein unfolding and aggregation.

To test the hypothesis, lyophilized α-chymotrypsin powder was incubated at various relative humidities for different time intervals. Formation of buffer insoluble and soluble aggregates was monitored for these samples. To detect unfolding/refolding events occurring to the solid protein, these were analyzed by FTIR and circular dichroism spectroscopy after incubation. Results showed that the protein forms aggregates upon storage in humidity chambers. Furthermore long-term storage of the proteins at increasing levels of residual moisture increases the formation of aggregates with this effect also increasing with storage time. Moreover results showed that the protein looses its native-like structure after storage for one week. This suggests that high moisture levels cause detrimental events in the protein such as, structural perturbations. This study has showed that residual moisture plays an important role in the denaturation and aggregation of protein. Previously published results revealed that the protein structural dynamics can be decreased after surface chemical modification with polymers by reducing protein-water contacts thus resulting in less degradation of the protein. To that effect, chemical modification with glycans (Dextran and lactose) was performed to test if glycosylation could decrease protein unfolding and aggregation. Initial results have shown a decrease in aggregation levels and an increase in residual activity in the solid-state upon the modification.

Program/Abstract# 119

On the role of protein structural dynamics in the catalytic activity and thermostability of serine protease subtilisin Carlsberg

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The effect of modulating the structural dynamics by incremental glycosylation on enzyme activity and thermostability has thus far only been investigated in detail for the serine protease α-chymotrypsin (for a recent review see Solá *et al*., *Cell. Mol. Life Sci.* 2007, 64(16), 2133–2152). Herein, we extend this type of study to a structurally unrelated serine protease, specifically, subtilisin Carlsberg (Pagán *et al*., *Biotechnol Bioeng* 2009**,** 103(1), 77–84).The protease was glycosylated with activated lactose and various lactose-subtilisin conjugates were obtained and characterized. Near UV-CD spectroscopy revealed that the tertiary structure was unaffected by the procedure. H/D exchange FT-IR spectroscopy was performed to assess the structural dynamics of the enzyme. It was found that increasing the level of glycosylation caused a linearly dependent reduction in structural dynamics.This led to an increase in thermostability and a decrease in the catalytic turnover rate for both, acylation and de-acylation step. These data highlight the possibility that a structural dynamics – activity relationship might be a phenomenon generally found in serine proteases.

Program/Abstract# 120

O-mannosyl glycosylation is involved in assembly of the basement membrane

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Congenital muscular dystrophies with brain malformations are a group of diseases that exhibit overmigration of neurons in the cerebral cortex. At least some are caused by genetic defects of O-mannosyl glycosylation. Earlier studies have demonstrated that disruptions of the pial basement membrane are the underlying causes of overmigration. Hypoglycosylation of α-dystroglycan, an extracellular matrix receptor, results in abolished interactions between α-dystroglycan and laminin. While disruptions of the pial basement membrane can theoretically be caused by its reduced strength, increased turnover, or reduced assembly, the results indicated that assembly of the basement membrane was defective due to diminished extracellular matrix−α-dystroglycan interactions. Defective assembly caused multiple disruptions of the pial basement membrane during rapid expansion of the developmental cerebral cortex. Overmigrated neurons disrupted the developing mesenchyme, resulting in ectopic fibroblasts in the cortex. Further studies suggested that these ectopic fibroblasts induced reactive astrogliosis.

Program/Abstract# 121

Comprehensive glycan analysis of Alpha-1-antitrypsin in hepatitis C induced liver cirrhosis and cancer

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We have previously reported changes in N-linked glycosylation with the development of liver cancer and identified many of those proteins containing altered glycan structures. To advance these studies we performed N-linked glycan analysis on one of these proteins, Alpha-1-Antitrypsin (A1AT) and completed a comprehensive study of the glycosylation of A1AT found in healthy subjects and patients with Hepatitis C induced liver cirrhosis and cancer. No significant changes in protein concentration were observed, however, we found an increase in tri-antennary (A3G3) glycan in patients with cirrhosis and an increase in tri-antennary glycan containing outer arm fucosylation in cancer. This is the first report of the stepwise changes glycosylation on A1AT with the progression from liver cirrhosis to cancer. Initially, we preformed a Lectin FLISA using AAL lectin, specific for core and outer arm fucosylation in over 500 patients with liver disease. In this analysis AAL reactive AAT was able to detect HCC with a sensitivity of 85% and a specificity of 75%, which was greater than that observed with the current marker of HCC, AFP.

Program/Abstract# 122

Genetic alteration of mannosylphospho dolichol synthase differentially regulates angiogenesis

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Mannosylphospho dolichol synthase (DPMS), a 31 kDa phosphoprotein is essential for the synthesis of lipid-linked oligosaccharide (LLO; $Glc_3Man_9GlcNAc_2$-PP-Dol), a pre-requisite for asparagine-linked (N-linked) protein glycosylation. Earlier observations from our laboratory have suggested that up-regulation of DPMS activity plays an important role in capillary endothelial cell proliferation and consequently in angiogenesis. To verify independently the impact of DPMS in angiogenesis we have isolated stable cell lines over-expressing DPMS and after silencing by siRNA. DPMS expression in these cells has been confirmed by fluorescence microscopy, western blotting, qRT-PCR and measuring the catalytic activity. All detected elevated DPMS level in over-expressing clone but is down-regulated in cells silenced with siRNA. Fluorescence microscopy with WGA supported high expression of complex glycans on the cell surface over-expressing DPMS and is considerably reduced in cells DPMS silenced with siRNA. Western blotting with Con A and WGA also support this observation. Overexpressing DPMS clone proliferates faster compared to the cells whose DPMS has been silenced with siRNA. This is corroborated with the cell proliferative antigens Bcl-2 and PCNA expression monitored by western blotting and/or by qRT-PCR. In wound healing assay DPMS overexpression facilitated faster cell migration than its siRNA clone. Thus, DPMS exerts influence on capillary endothelial cell proliferation and may serve as a modulator of angiogenesis. We, therefore, conclude that DPMS is a potential target for developing anti-angiogenic therapeutics treating breast cancer. Supported by the grants from Susan G. Komen for Cure BCTR0600582 (DKB) and G12-RR03035 (KB).

Program/Abstract# 123

Study on novel α-*N*-acetylglucosaminidases: hydrolytic activity and substrate specificity

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Alpha-linked GlcNAc is found in heparin, heparan sulfate, *O*-glycans on Class III mucin secreted from the gastric and the duodenal mucous-cells of higher animals, lipopolysaccharides of some enterobacteria, and glycophosphoceramides of plants. α-*N*-Acetylglucosaminidases (αGNase) classified into glycosidase family GH89 is useful tool for elucidation of the detailed structure and biological function of αGlcNAc-containing oligosaccharides. However, the enzyme is poorly known except for mammalian αGNases, which are responsible for degradation of heparan sulfate in the lysosome. In this study, we investigated the biological significance of the bacterial αGNase from *Clostridium perfringens* strain 13 (αGNaseC) having tandem repeated CBMs (carbohydrate binding modules). We assessed the detailed hydrolytic activity of bacterial αGNases against natural or natural-like saccharides as a substrate. The Km values of full length αGNaseC and CBM-truncated αGNaseC for GlcNAc1,4Galβ-*O*-*p*NP were 0.28 and 0.20 mM, respectively, whereas the released GlcNAc was significantly increased when the full length αGNaseC was added in the reaction mixture containing Class III mucin. This result shows that the CBMs serve to bind or approach to non-reducing end galactose in *O*-glycan of mucin. Furthermore, we will discuss the difference of the substrate specificities between human αGNase (NAGLU) and bacterial αGNases.

Program/Abstract# 124

Simultaneous determination of nucleotide sugars with ion-pair reversed-phase HPLC and LC-ESI-MS

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Glycosylation is regulated by various factors such as nucleotide sugars, their transporters, glycosyltransferases, and glycosidases. Nucleotide sugars are donor substrates of glycosyltransferases and their availability and localization regulate glycosylation levels.

We have recently established a method for simultaneous determination of a series of nucleotide sugars involved in glycosylation. A mixture of eight nucleotide sugars and relevant nucleotides was perfectly separated on a C18 column and were detected by UV detector. The developed method enabled us to determine the nucleotide sugars in cell extracts in a single run. To study how the cellular levels of nucleotide sugars are regulated during cancer progression, we determined their levels in breast cancer cells and in normal epithelial cells. The levels of UDP-GlcNAc, UDP-GalNAc, GDP-Fuc, and UDP-GlcUA were significantly increased in breast cancer cells. We also developed the high-sensitive method combined with ESI-MS, for determining nucleotides sugars in organelle and monitoring cellular dynamics of monosaccharides. This method gave 80-folds high sensitive analysis compared with UV detection and enabled us to simultaneously determine those in cell extracts (5 x 10^4 cells).

In our newly developed method we could be able to determine the levels of most of all kinds of nucleotide sugars in cells, which enable us to show an overview of cellular glycoslation status.

Program/Abstract# 125

Monoclonal antibodies to human polypeptide GalNac-T14

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Polypeptide GalNAc-transferase T14 has been implicated in sensitivity to death-receptor DR4 and DR5 induced apoptosis by Apo2L/TRAIL. The mechanism for this has not been fully elucidated, but evidence suggests that GalNAc-T14 regulates the function of the DR4 and 5 receptors through O-glycosylation and that altered expression of GalNAc-T14 in cancer is one mechanism for resistance to apoptotic signaling. GalNAc-T14 is one isoform of a family of 20 GalNAc-transferases involved in initiation of mucin-type O-glycosylation. We are developing a panel of monoclonal antibodies to distinct isoforms of this family to be able discern specific functions of each member. Here we report production of two mouse IgG1 monoclonal antibodies to human GalNAc-T14 using insect cell produced recombinant soluble enzyme for immunization. MAb CCG3D2T14 was directed to a native conformational epitope, while MAb CCG5G10T14 was directed to denatured epitopes and functioned in SDS-PAGE Western blot analysis. Immunohistology analysis with CCG3D2T14 showed an expression pattern in agreement with previous Northern analysis with preferential expression in kidney. GalNAc-T14 was expressed in colon carcinomas whereas no expression was found in breast tumours. A similar distribution was found in human cancer cell lines with strong expression in A704 (kidney adenocarcinoma), HT29 (colorectal adenocarcinoma) and HL60 (peripheral blood promyelocytic), whereas no expression was found in other cancer cell lines (*e.g.* breast cancer cell line T47D (breast ductal carcinoma). MAb CCG5G10T14 labelled a single band in Western blot analysis in agreement with the predicted mw. The monoclonal antibodies should be useful tools to further

aid in deciphering biological functions of the GalNAc-T14 isoform.

Program/Abstract# 126

N-Glycans modulate the activation of a common cytokine signal transducer, gp130, in mouse embryonic neural stem cells

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gp130 is a ubiquitously expressed transmembrane glycoprotein and signal transducer of interleukin 6 family of cytokines. In neural stem cells, undifferentiated neural cells endowed with a high potential for proliferation and the capacity for self-renewal with retention of multipotency, gp130 is involved in maintenance of the self-renewal ability and induction of the astrocyte differentiation. gp130 has 11 potential N-glycosylation sites in the extracellular domain, and 9 of them are actually N-glycosylated. However, the structure and functional role of the carbohydrate chains carried by gp130 are totally unknown. In this study, we examined the functional role of N-glycans of gp130 in mouse neuroepithelial cells which are known to be rich in neural stem cells. In neuroepithelial cells treated with tunicamycin, an N-glycosylation inhibitor, unglycosylated form of gp130 was detected. The unglycosylated gp130 was not phosphorylated in response to the stimulation with leukemia inhibitory factor, an interleukin 6 family of cytokine. Although the unglycosylated gp130 was found to be expressed on the cell surface, it could not form a heterodimer with leukemia inhibitory factor receptor. These results suggest that N-glycans are required for the activation, but not for the translocation, of gp130 in neuroepithelial cells.

Program/Abstract# 127

Sugars, stable isotopes, and spectrometry: new methods for the analysis of carbohydrate metabolism

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Structural analysis of carbohydrates involves three parameters: composition, linkage, and conformation, and tends to rely on the various forms of two techniques; mass spectrometry (MS) and nuclear magnetic resonance (NMR) spectroscopy.These techniques are enhanced and extended by the use of stable isotopes, especially ^{13}C and ^{2}H.Because these isotopes can be selectively detected they are typically used in metabolic pulse-chase experiments similar to radiolabels.This presentation will focus on three newer aspects: 1. GC-MS analysis of global metabolic labeling from ^{13}C precursor sugars; 2. Hydrogen-deuterium exchange mass spectrometry (HX-MS) for oligosaccharide conformational studies; and 3. Stable isotope-enhance diffusion-ordered ^{13}C-NMR spectroscopy (SIE-DOSY ^{13}C-NMR). Applications and advantages of these techniques for the study of microbial carbohydrate metabolism will be presented.

Program/Abstract# 128

Immobilization of *Helicobacter pylori* α1,3-fucosyltransferase on magnetic beads *via* C-terminal membrane anchoring region

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Chemical and Enzymatic (enzymatic-assisted chemical) synthesis of glycoconjugates is a promising method for the construction of those library. Enzymatic glycosylation often takes place stereo- and regioselectively under mild reaction conditions without any elaborate procedures such as protection and deprotection of hydroxyl groups in chemical glycosylation. Preparation of glycosyltransfearse, however, requires extremely laborious and time-consuming processes that entail molecular cloning, expression, multistep purification, and concentration. The development of efficient immobilization method of glycosyltransferase should enable us to gain powerful tool for glycobiology. The C-terminal region of *Helicobacter pylori*α1,3-fucosyltransferase (FucT) have 2-10 repeats of 7 amino acids (known as heptad repeats), which connect the catalytic domain with the C-terminal putative amphipathic α-helices. Here we present the immobilization of *Helicobacter pylori*α1,3-FucT on membrane-mimetic magnetic beads *via* the C-terminal membrane anchoring region. We succeeded in preparing the full-length form α1,3-FucT with high solubility (13 mg/mL) and activity (16 U/mL) by improving induction condition and buffer composition in purification processes. The purified enzyme was immobilized on the phosphoryl choline coated magnetic beads. Combined use of the α1,3-FucT-immobilized magnetic beads, bacterial β1,3-GlcNAcT, and human β1,4-GalT allowed for the synthesis of various glycoconjugates having Lewis X structure. Interestingly, the immobilized enzyme as a biocatalyst for fucose extension of glycoconjugates containing poly-*N*-acetyllactosamine structure retained activity even after 50 times of recycling. This approach may be the general immobilization method of various glycosyltransferases.

Program/Abstract# 129

Automated N-glycan composition analysis with LC-MS/MS

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Introduction Compared to proteomic profiling and differentiation, glycan analysis still employs a lot of manual work and can be a burden with increasing number of spectra. The aim of this study is to ease the work by using in house developed glycomic software methods in combination with existing proteomic tools. The resulting workflow is targeted especially to glycan LC-MS/MS analytics and can be run with a minimal amount of human intervention. The final goal is to apply the developed methods to profile and differentiate stem cell surface glycans.

Methods Glycans are permethylated using a method described earlier [2] and analysed with LTQ Orbitrap XL instrument. M/z-values of potential glycans are extracted with Progenesis LC-MS expression analysis software (Nonlinear Dynamics Ltd) and MS^2 spectra are extracted with Mascot Distiller (Matrix Science Ltd). Next steps of the workflow are built on in house developed R function library. The steps include spectrum filtering, search of potential glycan compositions, matching theoretical fragments with MS^2 spectra, combining MS and MS^2 results and deconvolution. MS^2 matching includes a novel probability based scoring scheme (developed from the scheme described in [1]).

Results The methods were tested with a mixture of commercially available N-glycan structures. The workflow was shown to be able to decrease analysis time considerably.

The focus of the method is to identify glycan compositions. If detailed structure or linkage analysis is needed, the workflow can be used as a preprocess step prior the other analysis software, for example GlycoWorkbench [3].

References

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Program/Abstract# 130

Sequence analysis of endo-α-N-acetylgalactosaminidases

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Family GH101 of glycoside hydrolases includes retaining endo-α-*N*-acetylgalactosaminidases [EC 3.2.1.97] and their uncharacterized homologues. The 3D structure of its representative from *Streptococcus pneumoniae* has been solved recently (PDB, 3ECQ). GH101 domains have a distorted $(\beta/\alpha)_8$-barrel structure. We have revealed 99 non-identical protein sequences of GH101 domains from GenPept database using the blast algorithm. They represent 21 genera of bacteria. We applied the PSI Protein Classifier program to analyze the order of sequence appearance during the first round of searches by PSI-BLAST, depending on the query. This analysis allowed us to distinguish six subfamilies in the GH101 family. Phylogenetic analysis of the GH101 family suggests the monophyletic origin of each subfamily. Most GH101-containing proteins have several additional common domains. Based on the conserved domain structure and presence of three invariant catalytically essential residues we consider the same enzymatic function for all proteins of the GH101 family. Iterative screening of the protein database by PSI-BLAST revealed the closest relationship of GH101 domains with uncharacterized protein domains, representing two new protein families. Proteins with accession numbers AAN24642.1 and EDM96541.1 are their representatives. More distant similarity was found with some proteins from GH13, GH20, GH27, GH29, GH31, GH36, GH66, GH97, COG1306, and COG1649 families. The closest of them is GH13, which is the biggest family among glycoside hydrolase catalytic domains. Nucleophile and proton donor of GH13 and GH101 proteins are located in the homologous positions. Using AAN24642.1 and EDM96541.1 as a query allowed us to reveal relationship with several other enzymatically uncharacterized protein families.

Program/Abstract# 131

Glycosaminoglycan-protein interactions in innate immunity and inflammation

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Discrimination of host from non-host by the innate immune system is believed to occur via the interaction of complement factor H (CFH) with polyanionic structures on host surfaces. A polymorphism (Y402H) in the complement factor H (CFH) gene has been associated with Age-related Macular Degeneration (AMD), which is the main cause of blindness in the industrialized world, where we have shown previously that this coding change (from a tyrosine to histidine residue) alters the binding properties of CFH for sulfated glycosaminoglycans (sGAG). We have hypothesized that this difference could affect the localization of CFH and thus the suppression of complement activation by CFH in host tissues; this is consistent with dysregulation of the complement pathway early in the disease pathology. We have recently demonstrated that the Y402H polymorphism profoundly affects CFH binding to sites within normal human macula, due to the differential recognition of sGAG by the two variants. Notably, the AMD-associated 402H CFH variant binds ~2–3-fold less well than 402Y to heparan sulfate and dermatan sulfate structures within Bruch's basement membrane, while both allotypes exhibit

a similar level of binding to the RPE; endogenous CFH was found to be present at both of these sites. Importantly, AMD is preceded by the formation of deposits, termed drusen, that accumulate between the Bruch's membrane and the retinal pigment epithelium (RPE), disrupting the neurosensory retina and promoting inflammation. Therefore, we propose that this fundamental difference in binding-site recognition exhibited by the 402H and 402Y variants of CFH within the macular microenvironment would adversely affect complement regulation and thus contribute to the development and progression of AMD.

Program/Abstract# 132

Cancer biomarker discovery – Exploring the O-Glycoproteome for biomarkers

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Auto-antibodies to cancer antigens hold great promise as sensitive amplified biomarkers for early detection of cancer. Most high through-put strategies to discover such auto-antibodies fail to allow identification of antibodies specific for cancer-associated posttranslational modified (PTM) variants of normal proteins. Yet aberrantly processed proteins are likely auto-antibody targets. Due to the complex regulation of mucin-type O-glycosylation aberrant O-glycoproteins may be a fruitful PTM-Ome to data-mine for such auto-antibodies. The cell membrane mucin MUC1 is over-expressed and aberrantly glycosylated in many cancers and we have used this as a model to evaluate the potential of natural cancer-induced auto-antibodies to aberrant O-glycoforms of MUC1 as sensitive diagnostic biomarkers of disease. We first demonstrated that circulating mucins in cancer patients are limited to "normal" glycoforms using an antibody based glycoprofiling ELISA. We then developed an O-glycopeptide microarray and used this to demonstrate detection of vaccine induced IgG auto-antibodies to MUC1 aberrant O-glycopeptide epitopes. Finally, screening of cancer sera led to identification of distinct aberrant MUC1 O-glycopeptide epitopes that are targeted by cancer-associated IgG auto-antibodies. The results suggest that auto-antibodies to aberrant O-glycopeptide epitopes may represent a fruitful and previously unaddressed source of sensitive biomarkers for early detection of cancer.

Program/Abstract# 133

O-glycan functions in development and cancer

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Aberrant glycosylation is associated with many developmental defects and diseases, including cancer. A major type of protein glycosylation is controlled by modification of the precursor antigen termed Tn (GalNAc α 1-Ser/Thr), which is not expressed in normal human and murine tissues. Expression of the Tn antigen and SialylTn antigen NeuAc α 2-6GalNAcα1-Ser/Thr is seen in at least 60~70% of human carcinomas, including breast, colon, cervix, ovary and pancreas cancer. The Tn antigen is normally modified by the Core 1 β3GalT (T-synthase), a key enzyme in mucin type O-glycan biosynthesis, to form the Core 1 structure Gal β1-3GalNAc α-Ser/Thr (T-antigen), which is expressed by almost all cells in humans and mice. Other enzymes such as the Core 3 β3GlcNAcT can also modify the Tn antigen to produce the Core 3 structure GlcNAc β1-3GalNAc α1-Ser/Thr, which is expressed mainly in the gastrointestinal tract. Both Core 1 and Core 3 glycans are usually modified to form complex O-glycans. The key regulator of the T-synthase is the unique molecular chaperone Cosmc (Core 1 β3GalT-specific molecular chaperone). *Cosmc* is located on the X-chromosome (Xq24), and thus a single mutation in the *Cosmc* gene in either male or female cells can result in a complete loss-of-function for the T-synthase. Cosmc is an ER-localized type-II membrane protein that prevents aggregation/proteasomal degradation of T-synthase. The expression of Tn and STn antigens in human tumors is associated with poor prognoses, but the molecular mechanism or genetic basis for their expression in human tumors is not well understood. This presentation will discuss evidence for acquired and engineered mutations in Cosmc and alterations in O-glycan biosynthesis associated with defects in development and in cancer.

Program/Abstract# 134

Biosynthesis of glycolipids and its regulations in apoptotic carcinoma cells

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Eukaryotic plasma membranes maintain the distinction between a normal cell and a cancer cell. Biosynthetic steps of Ganglio-, Globo-, Muco-, and Lewis-a/X families of GSLs have been established in recent years. The glycosyltransferases (GLTs) catalyzing their synthesis have been characterized in Golgi-bodies. Very little is known about gene-regulations of these GLTs either during embryonic development or during metastatic processes. We know the complete biosynthetic pathways of GSLs during embryonic development or onset of oncogenic processes, but its regulation during apoptosis is unknown. Inhibitors of GLTs (L-PPMP and D-PDMP) and DNA (*cis*-platin) trigger apoptosis in Colo-205, SKBR-3, MCF-7, and MDA-468 through either intrinsic or extrinsic apoptotic pathways. These inhibitors regulate GLT gene expression post-translationally as well as post-transcriptionally. Apoptotic effects initiate activation of Caspases (-3, -8, and -9). Using novel DNA-microarrays specifically designed for screening over 340 Glyco-related genes, transcriptional-regulation of several glycosyltransferases involved in the biosyntheses of Sialo-Le^X and Sialo-Le^a (cancer cell surface antigens) was observed with L-PPMP. Down-regulation of GLT activities and up-regulation of some GLT mRNA suggest a tight regulation of these enzymes by signal transduction pathways. These apoptotic agents could be employed as a new generation of anti-cancer drugs. Proper drug delivery system (Liposome Magic Bullet containing cis-platin) may regulate GLT gene expression effectively at low doses.

Program/Abstract# 135

Bllod group Glycosyltransferases: structure and function of natural and unnatural mutants

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We are using the human blood group A and B synthesizing enzymes, GTA and GTB, as models for investigating structure-function relationships for retaining glycosyltransferase enzymes. GTA and GTB catalyze the transfer of GalNAc or Gal, respectively, from their corresponding UDP-donors, with retention of configuration of the transferred sugar, to αFuc (1→2)βGal-R acceptors. GTA and GTB are highly homologous enzymes that differ in only four of 354 amino acids, Arg176Gly, Gly235Ser, Leu266Met, Gly268Ala, which alter the donor specificity from UDP-GalNAc to UDP-Gal. The cloning of GTA and GTB codon optimized for expression in *E. coli* has yielded large quantities of protein for kinetic and X-ray structural studies. Key amino acid residues responsible for donor discrimination and acceptor binding have been identified. There are also two flexible regions in the enzymes, an internal loop and the last ten C-terminal residues that become ordered upon substrate binding. We are carrying out mutagenesis to determine the exact roles of residues in these flexible regions in substrate binding and turnover. We are also producing enzymes based on the 80 mutations in the ABO genes have been discovered in blood banking laboratories. These include enzymes with alterations in donor specificities including *cis*-AB enzymes capable of utilizing both UDP-GalNAc and UDP-Gal donors.

Program/Abstract# 136

Genetic and biochemical analysis of the biosynthesis and assembly of the glycan N-linked to flagellins of the archaeon Methanococcus maripaludis

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Archaeal flagella are unique motility structures that are very different in structure and assembly from bacterial flagella. *Methanococcus maripaludis* is a methanogenic archaeon that is highly flagellated. The flagella are composed of three flagellins, all of which are glycoproteins. A four sugar glycan, containing unusual sugars, was identified and shown to be attached in an N-linkage via N-acetylgalactosamine to multiple asparagines on all three flagellins. The glycosyltransferases involved in attaching the second, third and fourth sugars of the glycan were identified by creating inframe deletions and examining the effect on flagellin molecular weight by western blotting. Decreases in flagellin molecular weight were confirmed by mass spectroscopy analysis of purified flagella. Complementation of the deleted gene restored flagellin molecular weight to wildtype size. The oligosaccharyltransferase (a homolog of the eukaryotic Stt3 oligosaccharyltransferase) was also identified. Partial glycans were readily attached to flagellins. In addition, several genes involved in the modification of the third and fourth sugars of the glycan were identified by flagellin molecular weight shifts and mass spectroscopy. Interference with glycan assembly had unusual effects on flagella assembly and function. At least two sugars of the glycan were necessary in order for flagella to be assembled. Cells lacking the third and fourth sugars of the glycan were able to assemble flagella of normal appearance and number. However, mutants missing the fourth sugar were less motile in swarm assays than wildtype cells and cells lacking the third and fourth sugars of the glycan were less motile than cells lacking only the fourth sugar.

Program/Abstract# 137

Cyberinfrastructure for glycome research

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Advances in Cyberinfrastructure, particularly in grid computing, are changing the way research is conducted in all aspects of science and have led to the generation of seemingly limitless possibilities of national and international collaboration and sharing of data for research, education and training. One of the applications of this development has made it possible to glue together all the databases for collaboration that individual researchers develop and maintain for their research. This capability can be exploited for shared data utilization, data analysis and more robust data mining and visualization of data. Grid computing provides high performance computing to TeraGrid, an NSF funded program that supports a number of scientific projects. The cancer Biomedical Informatics Grid (caBIG) at the National Cancer Institute is enabling the research community to share data and knowledge on cancer and beyond. Our recent development on the grid enabled information network for childhood obesity surveillance is already making some progress. Grid computing is also enabling this author for high-throughput protein motif analysis in the glycosyltransferase family. Analysis of a protein motif provides a better understanding on many aspects of protein function, protein interaction, and gene/protein and organism evolution. It also reveals evolutionary relationships between protein sequences that are too distantly related. However, such analysis is inherently highly computationally intensive because of the exponential growth of the protein databases and the combinatorial number of ways in which protein motifs interact in protein-protein interaction network. We have used MotifNetwork environment, built on biologically grid-enabled workflows, to serve this purpose and show that there is no evolutionary relatedness between the mammalian and bacterial sialylmotifs. This and other such plausible roles of grid computing in glycome research will be discussed.

Program/Abstract# 138

The conversion of trehalose to glycogen: a new pathway for synthesis of glycogen

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Trehalose and glycogen are both important stores of glucose that can be called upon as needed for energy or for carbon and building blocks. In addition, trehalose is important in protecting cells from stress, as a regulator of carbohydrate metabolism and as a cell wall component. In mycobacteria, there are three pathways for synthesizing trehalose and two of them utilize glycogen as the glucose donor. In this report, we show that trehalose can also be converted to glycogen in a newly described pathway which involves conversion of trehalose to maltose by trehalose synthase (TreS), then phosphorylation of maltose by maltokinase to form maltose-1-phosphate, and finally transfer of maltose to glycogen by a maltosyltransferase. Our hypothesis is as follows: "When trehalose levels get too high, such as after a stress response, TreS can expedite the conversion of trehalose to glycogen. On the other hand when trehalose levels get dangerously low, TreS can catalyze the breakdown of glycogen to trehalose. Thus, TreS may function as a sensor of trehalose levels in these cells". Recent studies have focused on the purification and characterization of maltokinase and maltosyltransferase from *Mycobacterium smegmatis.* A maltokinase with similar properties has been reported in actinoplanes but appears to be a different gene. Maltosyltransferase is a newly described enzyme as part of a new glycogen biosynthetic pathway. Those studies are described here.

Program/Abstract# 139

Noncanonical Asn-linked glycosylation occurs on recombinant heat shock protein 60 secreted from Chinese hamster ovary cells

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HSP60 was recently revealed to localize in cell surface and extracellular space, participating in various physiological and pathological processes, especially as an endogenous danger signal by stimulating inflammatory process. However, the recombinant HSP60 protein previously employed was expressed and purified from Eschrichia coli that it may be contaminated with prokaryotic components including LPS. Here, the eukaryotic cell CHO-K1 was employed to produce soluble HSP60 via conventional secreted pathway, where the protein underwent N-glycosylation. Surprisingly, the glycosylation was not completely abolished by mutation of the all three despragines in N-X-T/S consensus sequence, which implies N-glycosylation possibly occurs on non-canonical sites in recombinant HSP60 secreted via conventional secretion pathway in eukaryotic cells.

Program/Abstract# 140

Functional and structural analysis reveals dual function on C-terminal [alpha] helix of Alg13 protein

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As one of the most frequent and common post-translational modifications, protein asparagines *N*-glycosylation is initiated with the biosynthesis of a highly conserved dolichol-linked oligosaccharide (LLO) $Glc_3Man_9GlcNAc_2$ on the cytoplasmic face of the endoplasmic reticulum (ER) and ends within the lumen. The second step of this process is catalyzed by an UDP-*N*-acetylglucosamine transferase that is comprised of two subunits, Alg13 and Alg14. The interaction between Alg13 and 14 is crucial for UDP-GlcNAc transferase activity so formation of the Alg13/14 complex is likely to play a key role in regulation of *N*-glycosylation. Using a combination of bioinformatics and molecular biological methods, we have undertaken a functional and structural analysis of yeast Alg13 and Alg14 proteins to elucidate the mechanism of their interaction. Our results demonstrated that (I) Mutational studies demonstrated a short C-terminal α helix of Alg13 is required for interaction with Alg14 and for enzyme activity. Electrostatic surface views of the modeled Alg13/14 complex suggest the presence of a hydrophobic cleft in Alg14 that provides a pocket for the Alg13 C-terminal α helix; (II) Co-immunoprecipitation assays confirmed the C-terminal three amino acids of Alg14 are required for maintaining the integrity of Alg13/Alg14 complex and this depends on their hydrophobicity; (III) Interestingly, protein stability assay indicated that degradation of cytosolic free Alg13 also require this C-terminal αhelix, which may serve as an autonomous degradation signal.

Program/Abstract# 141

LOX-1, as a receptor, cross-presents Hsp60-fused antigen on MHC class I molecules

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Human heat shock protein 60 (Hsp60) elicits a potent proinflammatory response in the innate immune system and therefore has been proposed as a danger signal of stressed or damaged cells to immune system. Previous studies have demonstrated CD14, TLR2, and TLR4 as mediators of signaling, but probably not of binding. Although the receptor for Hsp60 has been proposed to be saturable and specific on macrophages, it is yet not well defined. In the present study we reported that LOX-1, as a receptor for Hsp60, could bind and internalize Hsp60 via the C-terminus of Hsp60. Yeast two-hybrid assay revealed that the second β-sheet containing the long loop region of LOX-1 played an important role in this interaction. Hsp60-induced LOX-1 signaling is not sufficient to activate downstream signaling and associated inflammatory responses in macrophages. However, Hsp60-fused OVA antigen could be cross-presented on MHC class I molecules via LOX-1. Taken together, these results demonstrate that LOX-1 functions as a receptor for Hsp60 and can cross-present Hsp60-fused antigen on MHC class I molecules.

Program/Abstract# 142

Highly pathogenic avian H5N1 viruses that acquire human receptor specificity

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H5N1 influenza A viruses have spread to numerous countries in Asia, Europe and Africa, infecting not only large numbers of poultry, but also an increasing number of humans, often with lethal effects. Human influenza A viruses bind predominantly sialic acid (SA)α2-6Galatose (Gal) linkages (2-6), whereas bird viruses bind SAα2-3Gal (2-3) predominantly. A conversion from SA2-3Gal to SA2-6Gal recognition is thought to be one of the changes that must occur before avian influenza viruses can replicate efficiently in humans and acquire the potential to cause a pandemic. By identifying mutations in the receptor-binding HA molecules that would enable avian H5N1 viruses to recognize human-type host cell receptors, it may be possible to predict the emergence of pandemic viruses. Here we show the Receptor binding profile of H5N1 viruses and some H5N1 viruses isolated from humans can bind to human type receptors.

Program/Abstract# 143

Structural basis of carbohydrate receptor recognition by viruses

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The interaction between a virus and a host cell receptor represents the first step of a complex process that eventually cumulates in an infection of the cell. Viruses must not only be able to specifically attach to cells in order to initiate entry, but their newly-formed progeny must also be able to release themselves from the cell membrane after an infection. As a result, attachment and release processes depend on precisely regulated contacts and affinities between viral proteins and their cognate ligands at the cell surface. Subtle changes in either the receptor or the virus coat protein can easily alter ligand binding properties, leading to changes in specificity, pathogenicity, and tropism. Frequently, viruses use more than one receptor to engage the host cell, and conformational changes often accompany the engagement process. Key unanswered questions are: (i) how does the engagement of one receptor influence the binding site and thus engagement of another

receptor; (ii) what are the relative affinities for the different receptors, and (iii) can changes in these binding sites result in altered virus properties such as tropism or spread. Our research seeks to answer these question through structure-function analyses of complexes between viral proteins and their glycan receptors. I will present recent data on our analysis of receptor binding properties of polyomaviruses and reoviruses, both of which use carbohydrates as receptors. Structural insights into ligand binding as well as data from affinity measurements will be presented. Our results contribute to the development of general principles of virus-receptor interactions, help to establish new models of tropism, and reveal basic mechanisms of pathogenesis. Elucidation of these unifying themes may lead to identification of new targets for broadly effective antiviral therapeutics that are based on carbohydrate receptor ligands.

Program/Abstract# 144

Novel fucose binding lectin domain from the human pathogen *Bukholderia cenocepacia*

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Burkholderia cenocepacia is a Gram-negative bacterium which belongs to the *Burkholderia cepacia* complex. This bacterium is dangerous for immunocompromised people especially to patients suffering cystic fibrosis and chronic granulomatous diseases. Over 20 percent of infections lead to a so called "Cepacia syndrome" resulting in aspiratory failure with fatal consequences. Chronic pulmonary colonization by opportunistic bacteria (*Pseudomonas aeruginosa*, *B. cenocepacia* and other members of so-called *B. cepacia* complex) is the leading cause of death among cystic fibrosis patients. Bacterial lectins can mediate recognition and adhesion to host cells so that they could be important virulence factors. Four genes coding proteins homologous to fucose-binding lectin PA-IIL, one of the *P. aeruginosa* virulent factors, have been identified in the genome of *B. cenocepacia* [1]. All of them were prepared in recombinant form showing their carbohydrate-binding abilities.

The contribution is focused on the BC-IIL-C lectin, a 271 amino acid protein, that displays affinity for both fucosylated and mannosylated saccharides. Separate cloning of N- and C-terminal parts of the protein showed that while C-terminal part, which is homologous to PA-IIL, displays narrow specificity for mannosylated oligosaccharides, N-terminal part represent a novel lectin domain with strict specificity towards fucosylated oligosaccharides. The two domains have been separately analyzed through cloning, glycan array, X-ray, surface plasmon resonance and isothermal titration microcalorimetry with human relevant saccharides. The N-term domain is a novel trimeric fucose-binding lectin, which carbohydrate-binding properties could be involved in microbial pathogenesis.

[1] Lameignere *et al*., Biochem. J. 411, 307, 2008

Program/Abstract# 145

Purification of glycophorin C and chracterization of its N - glycosidic chain - receptor for Plasmodium falciparum antigen EBA – 140

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Invasion of erythrocytes by the malaria parasite is a multistep process involving several specific interactions between receptors on the red blood cells and parasite ligands. Glycophorin C (GPC), a minor red blood cell glycoprotein was identified as a receptor for EBA-140 protein, a homologous to EBA-175, ligand of *P. falciparum.* The binding of EBA-140 antigen to GPC was sialic acid dependent and inolved its peptide backbone, similarly as in GPA – EBA-175 ligand interaction (1). However, in contradiction to GPA, the receptor site on GPC consists of its N-linked oligosaccharide chain as a crucial component (2). Therefore, it seems to be very resonable to identify, an unknown yet, structure of the N-glycan of GPC.

Due to of small amount of GPC in erythrocyte membranes and excess of GPA with which GPC agregates, obtaining pure GPC in amount sufficient for structural studies presents a problem. Pure GPC was obtained by the Sephadex G-200 chromatography of crude glycophorin in the presence of SDS, followed by SDS-PAGE of GPC – containing fraction and electroelution of a single GPC band. GPC obtained by this procedure was free of GPA that was confirmed in Western blotting using monoclonal antibodies with anti-GPC and anti-GPA specificity.

The N-glycosidic chain of GPC was characterized using several biotinylated lectins with defined specificity towards N-glycan moieties. The pattern of binding lectins from: *Griffonia simplicifolia, Canavalia ensiformis, Phaseolus vulgaris and Galantus nivalis* suggested the presence of complex type N- glycan. For mass spectrometry studies the N-glycan released by hydrazynolysis of GPC was used. The preliminary data obtained for GPC N-glycan indicated its structure to be similar to that found in GPA.

Program/Abstract# 146

An inhibitor of galactosyltransferases alters the cell surface properties of lung cancer cells

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The glycosylation of cell surfaces plays a major role in the interactions between cells and their environment. Thus agents that can modify cellular glycosylation are expected to alter cell surface functions. Cystic fibrosis patients suffer from lung infections with *Pseudomonas aeruginosa* and *Burkholderia cenocepacia*. Attachment of these bacteria to cell surface carbohydrates and mucins is a critical step in the infectious process, and glycan modification may reduce bacterial adhesion. Here, we investigated the effects of 2-naphthyl 2-butanamido-2-deoxy-1-thio-beta-D-glucopyranoside (compound 612), a potent inhibitor of Gal-transferases, in airway cell cultures and cell homogenates *in vitro*. We showed that compound 612 blocked the transfer of Gal to GlcNAcβ-benzyl in lung cell homogenates. Compound 612 added to cell cultures in phospholipid vescicles also increased binding of wheat germ agglutinin and decreased binding of ricin and *Maackia amurensis* lectin II binding to airway cancer cells A549 and H292. Compound 612 did not induce apoptosis, and minor effects on viability, morphology and proliferation of cells were seen. The adhesion of [^{3}H]Glc-labeled *Pseudomonas aeruginosa* bacteria to A549 and H292 cells was inhibited by pretreatment of cells with compound 612 as well as by a number of competing mono- and disaccharides. The binding of *Burkholderia cenocepacia* was inhibited by tunicamycin treatment of cells and increased after neuraminidase treatment. These results suggest the usefulness of Gal-transferase inhibitors in re-engineering the adhesive properties of lung cell surfaces and in understanding the interactions of pathogenic bacteria with cultured cells. This work was funded by the Canadian Cystic Fibrosis Foundation.

Program/Abstract# 147

Human noroviruses recognize α1,2-fucosylated glycosphingolipids on thin-layer chromatograms and in supported lipid bilayers

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Norovirus is recognized as the major cause of outbreaks of gastroenteritis world-wide. Susceptibility to human norovirus infection has been linked to secretor status and virus-like particles (VLPs) have been demonstrated to bind to glycoproteins carrying ABH antigens. In this study we demonstrate that norovirus VLPs also bind to ABH active glycosphingolipids (GSLs). Using a chromatogram binding assay we show that VLPs from the Norwalk (genogroup (G) I.1) and the Dijon (GII.4) strains bind to several α1,2-fucosylated type 1 chain GSLs. The Norwalk strain did not recognize the blood group B active GSLs B type 1 or BLe^{b}. In addition, the Norwalk virus was demonstrated to bind to type 2 chain GSLs carrying A and H but not B antigens. VLP binding to GSLs incorporated in laterally fluid supported lipid bilayers was studied using quartz crystal microbalance with dissipation (QCM-D) monitoring. In analogy with the results from the chromatogram binding assay both VLPs bound to bilayers containing H type 1 GSLs, whereas no binding was observed to bilayers containing Le^{a}. The binding kinetics was monitored in real time in stationary reaction chambers. Experiments using bilayers with varying concentrations of H type 1 and Le^{a}, with the total GSL concentration constant at 10 wt%, showed that binding was only dependent on H type 1 concentrations. Both VLPs showed a threshold concentration of H type 1 below which no binding was observable. The threshold was one order of magnitude higher for the Dijon strain demonstrating that interaction with a larger number of GSLs was needed for the binding of the Dijon strain. We propose that VLPs initially bind only a few GSLs but the binding is subsequently strengthened by lateral diffusion of additional GSLs moving into the interaction area.

Program/Abstract# 148

A novel Shigella galactosyltransferase with unusual properties involved in B14 O-antigen synthesis

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The synthesis of O-antigenic glycan chains of lipopolysaccharides (LPS) in Gram negative bacteria is catalyzed by specific glycosyltransferases. The genes encoding these enzymes are found in O antigen gene clusters but most of the gene products have not yet been characterized. *Shigellaboydii* is a highly adapted bacterial pathogen that causes dysentery in humans. A putative galactosyl-

transferase (GalT) *wfeD* gene has been cloned from the *Shigellaboydii* B14 strain. We showed that the *wfeD* gene encodes a GalT that forms the Gal-beta-GlcNAc-alpha-linkage as the second step in B14 O-antigen synthesis. WfeD was expressed in *E.Coli* BL21 cells and the activity was characterized using UDP-[3H]Gal donor substrate and the synthetic GlcNAc-alpha- pyrophosphate- phenoxy-undecyl acceptor substrate. The resulting disaccharide product was analyzed by LC-MS, HPLC, NMR and galactosidase digestion. The enzyme has little sequence similarity with other functionally characterized GalTs, but possesses a number of characteristics as GalT, including a DXD motif. The substrate specificity indicates that the enzyme is specific for UDP-Gal and requires pyrophosphate in the acceptor. Not only the divalent metal ions Mn2+, but also Ni2+ and Pb2+ enhanced enzyme activity. Our study revealed that B14 beta4-GalT WfeD is a novel enzyme with unusual properties although the catalysis, overall structure and some properties show similarities to other bacterial or mammalian galactosyltransferases. This work enhances our understanding of O antigen synthesis and forms the basis for discovery of biologically applicable inhibitors of LPS biosynthesis which may be useful for the prevention or treatment of intestinal disease (Supported by NSERC and the Gastrointestinal Disease Research Unit of Queen's University).

Program/Abstract# 149

Integrated approach to glycomics

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Glycomics or the study of structure-function relationships of complex glycans has reshaped post-genomics biology. Glycans mediate fundamental biological functions via their specific interactions with a variety of proteins. The unique challenges facing glycomics arises from the heterogeneity and structural complexity of glycans and their multivalent interactions with the protein binding partners. Recognizing the importance of glycomics, large scale research initiatives such as the Consortium for Functional Glycomics (CFG) were established to address these challenges. Our efforts at the CFG have led to the development of a framework for integrating technologies and datasets in glycomics. This integrated approach has enabled us to decode the complex roles of glycans in important biological processes such as human adaptation of influenza A virus.

Program/Abstract# 150

Mimicking the carbohydrate-carbohydrate cell recognition in marine sponge cells

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For a long time, direct carbohydrate-carbohydrate interactions between glycan chains of glycoproteins, glycolipids, and proteoglycans have been underestimated, if not ignored. However, nowadays the number of reports on this subject is growing. Typical examples from the glycomedical field comprise the Le^x – Le^x, the GM3 – Gg3, the (Kdn)GM3 – Gg3, the Gal-Cer – sulfatide, and the GM3 – GlcNAc-terminated N-glycan carbohydrate interaction couples. Here, an example from the glycobiological field will be presented. The first step in marine sponge cell recognition and adhesion operates via a Ca^{2+}-dependent carbohydrate – carbohydrate interaction. For the species *Microciona prolifera*, a red beard marine sponge, the 200 kDa N-glycan (g-200) involved in the self-recognition is part of a proteoglycan-like macromolecular complex, the aggregation factor, with a molecular mass of 2 x 10^4kDa. One of the carbohydrate epitopes involved in the self-interaction is a sulfated disaccharide fragment, β-D-Glc*p*NAc3*S*-(1→3)-αL-Fuc*p*. The other one is a pyruvated trisaccharide fragment, β-D-Gal*p*4,6(*R*)Pyr-(1→4)-β-D-Glc*p*NAc-(1→3)-α-L-Fuc*p*. In an attempt to mimick the polyvalent g-200 glycan – g-200 glycan self-recognition on the di- and tri-saccharide level, both epitopes were synthesized and conjugated with bovine serum albumin, gold nanoparticles, and gold layers. The protein conjugates were used in UV and SPR experiments, the gold glyconanoparticles in TEM and NMR experiments, and the gold glycolayers in AFM experiments. In addition, molecular modeling calculations were carried out. It turned out that in the presence of 10 mM $CaCl_2$ the various di- and tri-saccharide results match completely those obtained on the polymer level for *M. prolifera* cells and aggregation-factor-coated beads.

Program/Abstract# 151

Computational prediction of influenza receptor specificity

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Hemagglutinin (HA) mediates attachment to and entry of influenza virus into host cells by binding to sialic acid receptors at the cell surface. Human influenza viruses preferentially bind to sialic acid linked to galactose by alpha-2,6 linkages; the main type found on the epithelial cells of the human upper respiratory tract. Avian viruses tend to bind to alpha-2,3 linkages that are found predominantly on avian intestinal epithelium. All influenza A viruses that have infected mammals emerged as some point from avian species. Changes in the amino acid sequence of HA can alter the sialic acid specificity of influenza viruses, with the change of one or two amino acids being sufficient to change the receptor binding specificity and affect interspecies transmission barriers.

We report the results of computational molecular dynamics (MD) simulations (using the GLYCAM force field) [1] of human and avian receptor – HA complexes, based on structural data for the human 1934 H1 influenza strain. The theoretical methods correctly predict that this H1 hemagglutinin is selective for human alpha-2,6 linkages and provide insight into the origin of the affinity differences, but also indicate limitations of current simulational and docking methods.

1. Kirschner, K.N., *et al*., GLYCAM06: A Generalizable Biomolecular Force Field. Carbohydrates. *J. Comput. Chem.*, (2008) *29*, 622–655.

Program/Abstract# 152

Conjugated tunicamycin nanoparticles and nanotubes for breast cancer therapy

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Over the past decade, significant research is under way to mitigate and eliminate breast cancer. Currently approved immunotherapeutics work inadequately when administered as a single agent and must be given in combination with other chemotherapeutic agents. Major drawbacks of such therapies include side effects, high treatment costs, and few long-term benefits. We have developed tunicamycin as a new generation anti-angiogenic glycotherapeutic to alleviate breast cancer. Tunicamycin, (a glucosamine-containing pyrimidine nucleoside) inhibits glucosaminyl-1-phosphate transferase activity competitively and a potent inhibitor of N-linked glycoproteins. When treated (i) capillary endothelial cells undergo cell cycle arrest followed by apoptosis due to unfolded protein response; and (ii) breast tumor growth in athymic nude mice is reduced by ~65% in one week. Nanostructures with sizes smaller than 100 nm and with tailored surface properties should be able to evade the immune system's clearing mechanisms long enough to reach targeted diseased tissues efficiently. To enhance the efficacy of tunicamycin, we have conjugated the drug with varying groups of nanomaterials, which include threonine-based nanotubes, gold nanoparticles and CdS quantum dots. The attachment of the drug to the nanoparticles was confirmed by FTIR spectroscopy and electron microscopy. Initial screening indicates a successful synthesis of functionally active tunicamycin nanoparticles. 20 nm gold–conjugated nanoparticles are localized in the endoplasmic reticulum of the cell. Supported in part by grants from Susan G Komen for Cure BCRT0600532 (DKB) and NIH-RISE 3R25GM075348-03 S1 (CJH).

Program/Abstract# 153

Glycosignature analysis of complex glycans

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The analysis of carbohydrate modifications on serum and transmembrane glycoproteins and glycolipids is rapidly gaining momentum in the discovery of novel biomarkers, quality control of biopharmaceutical drugs, and the development of new drugs. Current analytical techniques however have been slow in addressing this rapidly expanding need for systems level glycomics and glycoproteomics. This situation provides a strong incentive to address the lack of high-throughput large-scale technologies to decode the glycosylation patterns (glycosignatures) more rapidly and cost-effectively. Particularly valuable would be analytical tools with the capacity to scrutinize the whole glycomic profile "holistically" and on a systemic level.

Here we report a new technological platform capable of simple and efficient glycosignature identification via the use of putative surface-bound carbohydrate recognition elements (CREs) arranged in microarray format in high density. Contrary to the lectin microarray approach, in using randomized putative ligands, there is no inherent limitation to the formation of binding sites, while the novel luminescent glyconanoprobes ensure highest level of sensitivity. As an example, we demonstrated the use of this platform for glycoprofiling of heterogeneous bacterial polysaccharides derived from different G-

bacteria. The screening of the polysaccharides labeled with quantum dots and the analysis of data produced orthogonal sets of CREs specific to each of the polysaccharides. Furthermore, the CREs were able to bind to the polysaccharides of choice in solution and demonstrated significant bacterial growth inhibition activity in vivo, ultimately serving as a validation of this technological platform.

Program/Abstract# 154

Bacterial carbohydrate structure database version 3

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The role of access to the data on bacterial carbohydrates in biomedical and immunological research can hardly be overestimated. In contrast to genomics and proteomics, universal standards and computer-assisted tools in glycomics are still in the making. BCSDB, a database containing data on bacterial carbohydrates with known primary structure, has been developed and updated since 2005. Now we present BCSDB ver. 3, the result of the database architecture rearrangement done in 2009.

The database contains structural, taxonomical, bibliographical, NMR spectroscopic and other information on ~10000 bacterial carbohydrates and glycoconjugates, including glycoproteins and glycolipids. This coverage approaches nearly all structures published within this class before 2009. The source of data were CarbBank and manual processing of publications. The data consistency was controlled by automatic and manual verification, which differs BCSDB from the other projects in glycoinformatics.

The BCSDB-3 provides web-based user interface and programming gateways for automated data interchange with other databases. Users can search the database using fragments of structure, bibliography, taxonomical annotations, fragments of NMR spectra, common structural motifs. The partial integration with other projects in glycomics (Glycome DB, NCBI PubMed, NCBI Taxonomy) has been achieved. The unambiguous but nevertheless human-readable carbohydrate structure description language has been developed for this project and translation tools to/from other known glycan representations are provided.

BCSDB is freely available at http://www.glyco.ac.ru/bcsdb3/. This work was supported by the ISTC, Rus. Foundation for Basic Research, Rus. President grant program, Deutches Krebsforshungszentrum program.

Program/Abstract# 155

Japan consortium for glycobiology and glycotechnology database

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As a part of Life Science Integrated Database project implemented by Ministry of Education, Culture, Sports, Science and Technology (MEXT), we promote a project aiming at the consolidation and integration of all glycoscience-related databases in Japan as many as possible. We have consolidated data, owned by each research institute, in JCGGDB and developed a keywords or glycan structure search system across those consolidated DBs. By one search, this enabled easy access to multiple glycoscience databases having the key words or glycan structure. Also Glycan structure editor made a sophisticated search easier with its intuitive interface, providing a convenient search function. Moreover, technologies to reduce a retrieval time have realized stress-free response time searching about 30,000 glycan structures. We will keep developing basic technologies and driving the integration between glycoscience-related DBs including other research fields. We also have plans to produce contents in which everyone, even those who study a different discipline, can easily utilize the expertise of glycoscience and to build the integrated glycoprotein DB closely focused on glycosylation.
http://jcggdb.jp

Program/Abstract# 156

Nothing in sialobiology makes sense, except in the light of evolution

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All cells in nature display cell surface glycans, which are involved in host biological processes, but also provide

binding sites for pathogens. Thus, glycans are trapped in never-ending cycles of evolutionary "Red Queen" effects, with long-lived hosts evading their more rapidly evolving pathogens by changing glycan expression, without compromising their own survival. This helps to explain the structural variations of glycans in nature, which contribute to biological diversity. Sometimes, a single glycan type is eliminated in one evolutionary lineage, *e.g.*, the human-specific loss of the mammalian sialic acid Neu5Gc, which has affected human pathogen regimes. Many human pathogens also disguise themselves with human-like sialic acids (molecular mimicry) via convergent evolution, thus taking advantage of host Siglecs (sialic-acid binding immunoglobulin-like lectins), which are normally dedicated to recognizing "self', and dampening innate immunity. The resulting genetic and biochemical differences between humans and great apes in relation to sialic acids have implications for unusual features of human immunity. Also, incorporation of Neu5Gc into humans occurs from foods such as red meat, and a similar process contaminates biotherapeutic molecules and cells produced using animal-derived materials. As all humans have complement-fixing antibodies against Neu5Gc-containing glycans, this could explain red meat associations with disease, and reactions to some biotherapeutic products. Finally, we hypothesize that such antibodies might restrict transmission of enveloped viruses carrying surface Neu5Gc derived from other organisms, and that the original loss of Neu5Gc could have lead to changes in fertility, allowing sympatric speciation (collaboration with Pascal Gagneux).

Program/Abstract# 157

GPI-glycan remodeling by PGAP5 regulates transport of GPI-anchored proteins from the ER to the Golgi

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Protein modification by glycosylphosphatidylinositol (GPI) anchors is one of the highly conserved posttranslational modifications in eukaryotes. Approximately 150 proteins in mammalian cells are modified by GPI, which is vital for embryonic development, immune responses and neurogenesis. GPI biosynthesis and attachment to proteins are carried out on the endoplasmic reticulum (ER) membrane. Many genes involved in the GPI biosynthetic pathway have been characterized in mammalian cells, yeasts and trypanosomes. However, how GPI-anchored proteins (GPI-APs) are trafficked from the ER to the cell surface is poorly understood, but the GPI moiety has been postulated to function as a signal for sorting and transport. Here, we established mutant cell lines that were selectively defective in transport of GPI-APs from the ER to the Golgi. We identified a responsible gene, designated *PGAP5*(post-GPI-attachment to proteins 5). PGAP5 belongs to a dimetal-containing phosphoesterase family, and catalyzed the remodeling of the glycan moiety on GPI-APs: removal of a side-chain ethanolamine-phosphate attached to the second mannose of GPI. PGAP5 catalytic activity is a prerequisite for the efficient exit of GPI-APs from the ER. Our data demonstrate that GPI glycan acts as an ER-exit signal and suggest that glycan remodeling mediated by PGAP5 regulates GPI-AP transport in the early secretory pathway.

Program/Abstract# 158

N-linked glycosylation in plants; curse or blessing for whom?

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N-linked glycans play crucial rules in the biology of mammals. The N-glycans observed in plants are very similar to those found in mammals. Plants produce complex glycans, having a core with two N-acetylglucosamine residues as observed in mammals. In plants, however, this core is substituted by xylose and α1,3- instead of α1,6-linked fucose. Furthermore, plant glycoproteins lack the characteristic β1–4 linked galactose and sialic acid-containing glycans found in mammals. Strikingly, and in sharp contrast to in mammalian biology, the structural identity of the complex N-glycans in plants seem to play little role in their biology. Mutations that prevent the addition of plant specific xylose and fucose residues or even those that prevent complex glycan biosynthesis entirely, display very little or no phenotypes.

The observation that the structural identity of complex type N-glycans in plants seems rather irrelevant to their development, turns into a benefit when their glycosylation machinery requires engineering to develop plants as production host for glycosylated therapeutic proteins. Indeed, the glycosylation machinery of several plant species has successfully been adapted to increase their applicability to produce such drugs. Genes responsible for plant specific modifications have been inactivated and mammalian enzymes were introduced to promote addition of typical mammalian residues. Also mutations have been described that may increase homogeneity of the N-glycans attached to glycoprotein products.

For plants, N-glycans seem to be neither a Curse nor a Blessing; their role in development still largely needs to surface. For man, the flexibility of plant N-glycan biosyn-

thesis may turn into a Blessing when plants start to deliver glycoprotein drugs to patients.

Program/Abstract# 159

Isotopic labeling and NMR characterization of glycans on glycoproteins

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Glycans mediate a wide variety of protein-protein interactions, including many that are important to human health. In an effort to understand the physical basis for glycan recognition many studies have been directed at the characterization of conformational properties of isolated glycans interacting with receptor proteins. However, when the glycans involved in these interactions are covalently attached to glycoproteins, the nature of interaction and recognition can be much more complex. Glycoprotein residues near the sites of glycan attachment can participate in protein-protein interactions. Presentation of glycans on the surface of glycoproteins can also become a factor. NMR methods can, in principle, provide information on the structure and dynamics of glycans, even when they are parts of complex glycoprotein systems, however application to glycans on glycoproteins has been inhibited by a requirement for labeling with appropriate magnetically active isotopes. Progress on the development of techniques for remodeling and introducing isotopically labeled glycans to mammalian glycoproteins will be reported. The methods are initially targeted at sialic acid terminated glycans, and employ a combination of treatment with neuraminidases and sialyltransferases to remove native sialic acids and replace them with isotopically labeled forms. Sialic acid terminated glycans are important to interactions involving the activation and recruitment of immune cells. Among the proteins involved are siglecs and immunoglobulins. Applications to the characterization of glycans on these systems will be discussed.

Program/Abstract# 160

The CVNH family of lectins - structure, folding and sugar binding

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Cyanovirin-N (CV-N) is a cyanobacterial lectin that exhibits antiviral activity against HIV at low nanomolar concentration by interacting with high-mannose oligosaccharides on the virus surface envelope glycoprotein gp120. Three dimensional structures of wild type CV-N revealed either a monomer in solution or a domain-swapped dimer in the crystal, with the monomer comprising two independent carbohydrate binding sites that individually bind with micromolar affinity to di- and tri-mannoses. We determined solution NMR and crystal structures of CV-N variants and characterized their sugar binding properties and the basis for activity of wild-type and mutant CV-N. Recently, homologs of CV-N have been identified and we determined structures for three additional members of this family of lectins. CVNHs from *T. borchii, C. richardii,* and*N. crassa,* representing each of the three phylogenetic groups were selected. All proteins exhibit the same fold and the overall structures resemble that of the founding member of the family, CV-N, albeit with noteworthy differences in loop conformation and detailed local structure. Since no data was available regarding the proteins' function or their natural ligands we conducted extensive carbohydrate binding studies. The number and location of binding sites vary for the three proteins and different ligand specificities exist. Potential physiological roles for two family members, TbCVNH and NcCVNH, were probed in nutrition deprivation experiments that suggest a possible involvement of these proteins in life-stage related responses.

Program/Abstract# 161

Structural basis for differences in carbohydrate specificities among members of Jacalin Related Lectin (JRL) family

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Lectins are multivalent carbohydrate-binding proteins that recognize diverse sugar structures with high specificity. These proteins are found in all life-forms, however, the majority of lectins studied, till date, are those isolated from plants. On the basis of structure, plant lectins are classified into five families, of which one is the β-prism I family. Jacalin was the first protein described with this fold, which is now an established characteristic of Moraceae family lectins. Subsequently, X-ray crystallography revealed that some lectins from other families, such as Banlec, Calsepa and Heltuba, also display the same fold. All lectins exhibiting the β-prism I fold are now regarded as members of Jacalin related lectin (JRL) family. They are further divided into two sub-groups viz. a) mannose-specific and b) galactose-specific lectins.

In the present study two lectins, Jacalin and Artocarpin, both of which are isolated from jackfruit (*Artocarpus integrifolia*) have been compared. Both proteins are tetrameric, sharing 57% sequence identity; yet, Artocarpin is a non-glycosylated, mannose-specific protein with a single 149 aa long polypeptide chain subunit while Jacalin is a glycosylated, galactose-specific protein with a 133 aa heavy α chain and a 20aa light β chain. A comparison of the structures and sequences of all the known members of the JRL family revealed that the primary structural difference observed between the two sub-groups is the prevalent post-translational modification in galactose-specific lectins, where the monomeric subunit of the normally homo-multimeric proteins undergoes proteolysis to generate two polypeptide chains, one long and one short. Another significant observation was that the binding site of Artocarpin does not include any aromatic residues; on the other hand, there are four such residues present in Jacalin. These aromatic residues are involved in stacking interactions with the galactose moiety. A comparison of the sequences of all mannose-specific lectins (with known crystal structures) indicates the absence of aromatic residues near the carbohydrate molecule in all of them. This suggests that stacking interactions have a more significant role to play, than previously thought of, in the generation of carbohydrate specificity. Additionally, within the mannose-specific sub-group, we observed that the variation in extended carbohydrate affinities *i.e.* to higher manno-oligosaccharides can be generated via variation in length of the loops at the carbohydrate binding site. The interactions with the loop become important in the binding of the disaccharide and higher oligosaccharides and, at that level, the affinities of any two lectins for carbohydrates become different. This study therefore highlights the differences in carbohydrate binding specificities of β-prism I fold containing lectins and suggests a possible structural rationale behind the same.

Program/Abstract# 162

Auto-antibodies to glycans: repertoire, specificity and proposed role in immunity

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We have used printed glycan array (205 glycans) for profiling of antibodies (Ab) in sera of 106 healthy donors. Serum Ab interacted with ~50 human glycans. Apart from blood group-, xeno- and infection-related activities, we observed Ab to: P_1, P^k, H antigens, Le^C related glycans, 4'-O-sulfated LacNAc. Relatively high and uniform Ab binding to GalNAcα1-3Gal demonstrated absence of correlation with fucosylated form, GalNAcα1-3(Fucα1-2) Gal, – similarly to well known relationship between Galα1-3Gal and true, fucosylated blood group B antigen. The binding intensity to Galα1-3Galβ1-4GlcNAc xenoantigen was shown to be modest. Absence or very low binding was found against oligosialic acid, sialooligosaccharides except $SiaT_n$, type 2 backbone glycans such as Le^y, and biantennary N-chain. Intriguing, Ab are capable of recognizing the short inner core typical for glycolipids (–Galβ1-4Glc) and glycoproteins (–GalNAcα) as a fragment of bigger glycans. Group of 106 donors is representative enough to relate the mentioned immunoglobulins to natural antibodies (NAb) rather than to immune antibodies. Yet, when comparing NAb profiles, we observe both individual features and regularities. Notably, the regularities are predominant that was especially descriptive for comparison of affinity purified Ab; *i.e.* the antibodies were inspired by similar repertoire of immunogens. Parsing of revealed wide Ab repertoire in light of known structures of bacterial polysaccharides gives evidence that hypothesis about enterobacteria as driving force of anti-glycan NAb is not complete and should be added with at least a hypothesis on malignant cells, in order to explain the existence of Ab against core motifs, sulfated and other glycans.

Program/Abstract# 163

Chondroitin sulfate proteoglycan-mediated adherence of *Plasmodium falciparum*-infected red blood cells in human placenta

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Plasmodium falciparum is the causative agent of the most severe form of malaria. About 500 million people are infected annually worldwide and 1-2 million people die of malaria. In endemic areas, although immunity to malaria develops during childhood years, women become susceptible to malaria during pregnancy due to the sequestration of the parasite-infected red blood cells (IRBCs) in the placenta, leading poor fetal outcomes, and maternal morbidity and mortality. The chondroitin 4-sulfate (C4S) chains of uniquely low sulfated CSPGs are the host receptors and a *var* gene family parasite protein termed VAR2CSA is involved in IRBC binding in the placenta. Although many of the C4S structural elements involved in IRBC binding is known, how VAR2CSA interacts with C4S remains poorly understood. VAR2CSA consists of six distinct Duffy binding like (DBL) domains and it is suggested that several DBL domains could mediate IRBC binding independently, and that together they provide multivalency for effective IRBC binding. We expressed various DBL domains on the yeast cell surface

and found that none of the domains could support binding of yeast to placental CSPG. Several parasite proteins, including VAR2CSA, in the parasite lysates could bind to immobilized C4S. Microarray and QRT-PCR analyses revealed that, besides VAR2CSA, several parasite proteins are highly upregulated in C4S-adherent IRBCs compared to nonadherent IRBCs. Together these data suggest that VAR2CSA is necessary for IRBC binding to CSPG but it is not by itself sufficient, and that a protein complex is involved. Funded by AI45086 from NIAID, NIH.

Program/Abstract# 164

The role of carbohydrates in viral infections – A Nuclear Magnetic Resonance (NMR) study

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A detailed understanding of how viruses interact with glycan molecules on the human cell surface is essential for the development of novel broad-spectrum anti-viral drugs and vaccines. Nuclear Magnetic Resonance (NMR) is an ideal tool to study such interaction at an atomic level. This presentation comprises recent NMR results on the interaction of cell receptor determinants with influenza virus-like particles (VLPs) derived from the highly contagious H5N1 ('bird flu') strain (1) , interaction of gangliosides with rotavirus VP8* proteins (2) and whole rotavirus particles.

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Program/Abstract# 165

Binding of porcine submaxillary mucin analogs to lectins

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Isothermal titration microcalorimetry (ITC) and hemagglutination inhibition measurements demonstrate that a chemically and enzymatically prepared form of porcine submaxillary mucin that possesses ~2300 alfa-GalNAc residues (Tn-PSM) and a molecular mass of $\sim10^6$ daltons binds with $\sim10^6$-fold greater affinity to the soybean agglutinin (SBA), which is a GalNAc-specific lectin, than the corresponding monovalent carbohydrate GalNAc1-*O*-serine, which is the Tn cancer antigen [1]. Furthermore, the enzymatically derived 81 amino acid tandem repeat domain of Tn-PSM containing 23 GalNAc residues showed $\sim10^3$-fold enhanced affinity, while the enzymatically derived 40/41-mer cleavage product of the 81-mer containing 11–12 GalNAc residues showed $\sim10^2$-fold enhanced affinity. A natural carbohydrate decorated form of PSM (Fd-PSM) containing 40% of the core 1 blood group type A tetrasaccharide, and 58% peptide linked GalNAc1-*O*-Ser/Thr residues, with 45% of the peptide linked α-GalNAc residues linked $\alpha(2,6)$ to N-glycolylneuraminic acid, shows $\sim10^4$ enhanced affinity for SBA. *Vatairea macrocarpa* lectin (VML), which is also a GalNAc binding lectin, displays a similar pattern of binding to the four forms of PSM, although there are quantitative differences in its affinities as compared to SBA. The higher affinities of SBA and VML for Tn-PSM relative to Fd-PSM indicates the importance of carbohydrate composition and epitope density of mucins on their affinities for lectins. The higher affinities of SBA and VML for Tn-PSM relative to its two shorter chain analogs demonstrate that the length of a mucin polypeptide chain and hence total carbohydrate valence determines the affinities of the three Tn-PSM analogs. The results suggest a binding model in which lectin molecules "bind and jump" from carbohydrate epitope to epitope in the mucin analogs before dissociating. The large increases in affinity of the lectins for the mucins are due to very favorable entropies of binding that are associated with the bind and jump mechanism. Importantly, the mechanism of binding of lectins to mucins is similar to that for a variety of protein ligands binding to DNA [2]. Analysis also shows that high affinity lectin-mucin cross-linking interactions are driven by favorable entropy of binding that is associated with the bind and jump mechanism [3]. These findings have important implications for not only binding of lectins to multivalent carbohydrates and glycoproteins including mucins, but for other ligand-biopolymer interactions in general. Additional experiments are underway to provide further insight into the bind and jump mechanism of lectins with mucins.

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Program/Abstract# 166

Syndecan-4 regulates the cell-surface trafficking and activity of pro-fibrotic factor Transglutaminase-2

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Transglutaminase-2 (TG2) is a Ca^{2+}-dependent crosslinking enzyme involved in the post-translational modification of proteins via the formation of ε(γ-glutamyl)lysine isodipeptides. TG2 is externalised in the extracellular matrix (ECM) through an unconventional and not fully understood pathway. Under normal conditions, TG2-mediated modification of ECM modulates cell adhesion, proliferation and tissue repair, but under continuous cell insult higher expression and elevated extracellular trafficking of TG2 contribute to the pathogenesis of tissue scarring. Given the known affinity of TG2 for heparin, we hypothesised that heparan sulphate (HS) proteoglycans regulate TG2 trafficking and activity in the ECM.

We demonstrated that: (1) TG2 has a high affinity for HS (Kd~20 nM) and it associates directly to the HS chains of syndecan-4 (Sdc-4); (2) TG2 crosslinking activity at the cell surface relies on the presence of the HS chains of Sdc-4 and other HS proteoglycans; (3) HS-binding does not have a direct role in TG2 activity regulation, but the presence of intact Sdc-4 is essential for TG2 localisation at the cell surface. These findings indicate that Sdc-4 is involved in the cell-surface trafficking of TG2. Furthermore, biochemical analysis of kidneys from wild type and Sdc-4 null mice subjected to experimental renal fibrosis (unilateral ureteral obstruction) revealed that Sdc-4 regulates the release and activity of the pro-fibrotic factor TG2, indicating a possible co-operative role of TG2 and Sdc-4 in the development of kidney fibrosis.

Program/Abstract# 167

Probing carbohydrate-carbohydrate interactions with multivalent glycoconjugates

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Multivalent carbohydrate-carbohydrate interactions mediate cell adhesion, myelination, and modulate signal transduction. Studies of carbohydrate-carbohydrate interactions have used a variety of multivalent platforms for detecting and interrogating these interactions. These platforms include glycosylated dendrimers and fluorescent nanoparticles. Methods for their preparation and applications for the detection of carbohydrate-carbohydrate interactions between a) lactose and GM3; and b) galactose and sulfogalactose will be presented.

Program/Abstract# 168

Carbohydrate-mediated cell adhesion involved in pathogenic homing behaviors of T- and B-lymphocytes

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The role of cell surface glycans is becoming increasingly recognized as an important determinant in lymphocyte traffic and homing. We recently found that a sulfated and sialylated cell-adhesion glycan, $\alpha2\rightarrow3$ sialylated 6-sulfo Lewis X, is specifically expressed in a subset of skin-homing helper memory T cells, and serves as a ligand in selectin-mediated skin homing. The T cells expressing the glycan belonged to T-helper-2 lineage, and were central helper memory T cells, which are known to exhibit bi-directional homing behavior, traveling between the skin and lymph nodes. The central helper memory T cells are highly related to the intractability of bronchial asthma and atopic dermatitis. The cells escape local steroid therapies when they home to lymph nodes, and cause recurrence of the maladies when they later return to the skin or lung. Our clinical results indicated that patients with intractable bronchial asthma and atopic dermatitis have a significantly increased number of central helper memory T cells bearing the sulfated ligand in their peripheral blood. We recently found another sulfated/sialylated cell-adhesion glycan, $\alpha2\rightarrow6$ sialylated 6-sulfo LacNAc, on human peripheral B cells. The glycan serves as a preferred *cis*-ligand for CD22 in B cells, and masks binding activity of CD22 to exogenous *trans*-ligands. We propose that the expression level of this glycan regulates extravasation of B cells. Once the endogenous ligand disappears from CD22+B cells, such cells become capable of interacting with exogenous CD22 ligands on endothelial cells, as the glycan is strongly expressed on human endothelial cells. Our results indicated that malignant B cells in patients with leukemia and lymphoma have significantly decreased expression of the endogenous ligand, while retaining CD22 expression. The unmasked CD22 on such malignant B cells is expected to facilitate adhesion of the cells to vascular endothelium, and hence result in tissue infiltration of leukemia/lymphoma cells.

Program/Abstract# 169

Carbohydrate-carbohydrate interactions between myelin glycosphingolipids

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Myelin, the multilayered membrane surrounding nerve axons, is the only example of a membranous structure where contact between extracellular surfaces of membrane from the same cell occurs. The two major glycosphingolipids (GSLs) of myelin, galactosylceramide (GalC) and sulfatide (Sulf), can interact with each other by trans carbohydrate-carbohydrate interactions across apposed membranes. They may contact each other across apposed extracellular membranes, thus forming glycosynapses in myelin. Multivalent forms of these carbohydrates, GalC/Sulf-containing liposomes, galactose conjugated to albumin, or galactose and sulfated galactose conjugated to silica nanoparticles have been added to cultured oligodendrocytes (OLs) to mimic interactions which might occur between GalC and Sulf when OL membranes or the extracellular surfaces of myelin come into contact. These interactions between multivalent carbohydrate and the OL membrane cause redistribution of myelin GSLs and some proteins to the same membrane domains. They also cause depolymerization of the cytoskeleton, indicating that they cause transmission of a signal across the membrane. Their effects are similar to those of anti-GSL antibodies on OLs, shown by others, and are prevented by inhibition of GSL synthesis with fumonisin B1, supporting the conclusion that the multivalent carbohydrate interacts with GalC/Sulf in the OL membrane. Use of inhibitors revealed that signaling pathways known to regulate the cytoskeleton are involved. Participation of transient GalC/Sulf interactions in glycosynapses between apposed OL membranes or between the extracellular surfaces of mature myelin might signal compaction of myelin layers and facilitate communication of axonal signals throughout the multilayered myelin sheath.

Program/Abstract# 170

Carbohydrate tumor antigen vaccines using novel strategies

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The Thomsen-Friedenreich glycoantigen (TF-Ag), a carbohydrate tumor associated antigen, is differentially expressed on breast, colon, bladder, prostate and other carcinomas, and has been shown to play a role in metastasis. We hypothesize that antibody to this antigen may create a survival advantage for patients with TF-Ag expressing tumors by cytotoxicity or by blocking tumor cell adhesion and inhibiting metastasis. The innovation of this work is that novel constructs will be utilized to enhance the anti-glycopeptide response. Synthetic TF-Ag MUC-4 peptide and C3d N- terminal peptide constructs were prepared to target CD21+ splenic B cells and CD21+ follicular dendritic cells for an increased T-independent type 2 response and splenic memory B cell production, since CD21 is the C3d receptor and MUC-4 is a tumor associated peptide. The immune response was measured using TF-Ag BSA, asialofetuin, the various TF-Ag MUC-4 constructs and MUC-4 in enzyme immunoassays. Preliminary protection studies are underway. The significance of enhancing antibody production to this tumor associated antigen can be seen in the fact that monoclonal antibody specific for alpha-linked TF-Ag has been shown to decrease lung metastasis and extend survival time *in vivo* by blocking adhesion. This improved survival time is of particular interest because the monoclonal antibody was not cytotoxic, and additional experiments showed that the enhanced survival was due to blocking metastasis. Additional significance lies in the fact that even low levels of naturally formed antibody to TF-Ag is related to better prognosis, thus active immunization to cause production of anti-TF-Ag antibody in the patient should improve prognosis by creating a cytotoxic antibody which also blocks metastasis.

Program/Abstract# 171

Stability of human plasma N-glycome

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Nearly all plasma proteins are glycosylated and their glycan parts can exist in various structural forms, resulting in different glycoforms of the same molecule. Glycan heterogeneity was shown to be associated with numerous diseases and glycan analysis has a great diagnostic potential. Recently we reported high biological variability of human plasma N-glycome at the level of population. The observed variations were larger than changes reported to be associated with some diseases, thus it was of great importance to examine the temporal constancy of human N-glycome before routine implementation of techniques that determine glycosylation changes in diagnostic laboratories. Contrary to the presence of high variability of

the glycome at the level of population we observed very small variation in all glycan groups within the same individual, indicating very good temporal stability of the N-glycome. Even after a period of one year, changes were visible only in some individuals and for some glycan groups. Coefficients of variation between 1.6% and 11.4% were observed; with the average coefficient of variation of 5.6%. These variations were comparable to those observed when analytical procedure was tested for its precision. It appears that plasma N-glycome in healthy individual has very good stability, what implies that it is under significant genetic control. Changes observed in glycan profiles are consequence of environmental influences and physiologic responses and therefore carry good potential diagnostic value.

Program/Abstract# 172

A new route to labeled oligosaccharides via glycosyl azides in aqueous media

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The regioselective introduction of a chromophore into the anomeric position is an important technique for synthesis of labeled oligosaccharides. Glycosyl azides are useful intermediates because the azide group can readily be converted to stable moieties such as amides and 1,2,3-triazoles by Staudinger ligation and click reaction, respectively.
Recently, we have reported the direct synthesis of various β-glycosyl azides starting from unprotected sugars by the action of 2-chloroimidazolinium salt (DMC) in aqueous solution without using any protecting groups(1). This result suggests that the introduction of chromophore into oligosaccharides would be possible in aqueous media by the combined use of the glycosyl azide synthesis and the successive conversion to labeled glycosidic compounds. In the present study, we have prepared oligosaccharides having a 1,8-naphthalimide moiety as a fluorochrome at the reducing end via the corresponding glycosyl azides starting from a mixture of laminari-oligosaccharides as a model system.

Laminari-oligosaccharides mixture (DP=1-7) were reacted with DMC in the presence of sodium azide and diisopropylethylamine in water. The resulting reaction mixture was then treated with 4-ethynyl-*N*-ethyl-1,8-naphthalimide catalyzed by cupper(I) iodide in the presence of diisopropylethylamine, giving rise to a mixture of labeled oligosaccharides. The chromatogram of the reaction mixture showed that each labeled oligosaccharide was clearly identified as a separated peak, indicating that the present technique is useful for detection of oligosaccharides from an oligosaccharide mixture.

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Program/Abstract# 173

Immunological disorder analysis of polylactosmaine synthase-deficient mice

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Polylactosamine is carried on *N*-, *O*-glycans, and glycolipids. Polylactosamine structures are considered to be integral components serving as backbones for the carbohydrate structures on glycoproteins. To ivestigate *in vivo* function of polylactosamine, we analyzed polylactosamine synthase-deficient mice. We generated and examined beta-1,3-*N*-acetylglucosaminyltransferase 2-deficient (T2KO) mice. Glycan analysis demonstrated that the amount of polylactosamine chains on *N*-glycan was greatly reduced in the tissues of T2KO mice. We examined immunological responses in T2KO mice. This result demonstrated that T2KO lymphocytes showed hyperactivation via TCR/CD28 or BCR stimulation. Thus, polylactosamine chains on glycoproteins are the important factors determining thresholds for *in vitro* immunocyte activation. These results indicate that polylactosamine on *N*-glycans is a putative immune regulatory factor. In addition, we observed alterations of some carbohydrate antigens such as Lewis antigens keratan sulfates and etc., in T2KO tissues. T2KO mice showed lower responses in contact hypersensitivity assays *in vivo*. We found that a reduction of E-/P-selectin ligands expression on T2KO neutrophils, and identified that these selectin ligands on polylactosamine chains were carried on PSGL-1, CD44, and other molecules. Down-regulation of selectin ligands in T2KO neutrophils may explain the lower responsiveness of contact hypersensitivity.

This work was supported by New Energy and Industrial Technology Development Organization (NEDO) in Japan.

Program/Abstract# 174

Mapping the substrate binding subsites of Golgi α-Mannosidase II and implications for inhibitor development

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α-Mannosidase II (GMII) is a player in the N-glycosylation process in glycoprotein synthesis in the Golgi. This enzyme has been identified as a target for inhibition of glycosylation, with possible applicability to cancer metastasis and microbial infection. This talk will review our progress in studying the structure and function of this molecule, and ideas towards the design of specific inhibitors.

Two recent results will be reviewed. Firstly, we have isolated the enzyme structure in the crystal complexed with the full hepta-saccharide substrate. This has allowed us to visualize the details of three main binding sites, which are unique to GMII in comparison to other members of the GH38 enzyme family. Secondly, studies of 5-substituted swainsonine complexes have provided additional insights that will guide the design of multi-site fragment-based compounds that are hypothesized to selectively bind to GMII.

Program/Abstract# 175

Heparin upregulates mir-10b targeting Hoxd10 and inhibit angiogenesis

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Recent evidence suggests heparin and other glycosaminoglycans play a role in vascular endothelial cell function, as they are able to modulate the activities of angiogenic growth factors by facilitating the interaction with their receptor and promoting receptor activation. However, the role of heparin in constituted by multiple steps complex angiogenesis process has not been fully delineated or appreciated. Recent study has shown that microRNAs, a newly discovered class of small ribonucleotide-based regulators of gene expression is being implicated in angiogenesis. For example, mir-221 and mir-222 were found to affect angiogenic properties by targeting c-kit expression. Mir-130a was a regulator of the angiogenic phenotype of vascular endothelial cells largely through its ability to modulate the expression of GAX and HOXA5. We first examined the effect of heparin in HMEC-1 on down-regulation of mir-10b, which was reported positively regulates cell migration, invasion, and metastasis in breast cancer. In this report, we show that (a) heparin down-regulates mir-10b in HMEC-1. (b) mir-10b can recover HMEC-1 the capillary-tube formation inhibited by heparin on the matrigel assay. (c) mir-10b enhances endothelial cell angiogenesis by target hoxd10, which was reported over-expressing in human endothelial cells also failed to form new vessels. (d) heparin up-regulates HOXD10 in HMEC-1 and hek293. The data demonstrate new mechanism underlying heparin impairing angiogenesis.

Program/Abstract# 176

Tunicamycin inhibits angiogenesis and breast tumor growth by modulating the cell survival signal

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Angiogenesis is essential for breast cancer progression and metastasis. Our laboratory has established a dynamic relationship between asparagine-linked protein glycosylation and angiogenesis. These studies targeted mannosylphospho dolichol synthase (DPMS). We now ask if other dolichol cycle step is linked to the angiogenic process. We have used tunicamycin (a glucosamine-containing pyrimidine nucleoside), a competitive inhibitor of N-acetylglucosaminyl 1-phosphate transferase. The reduction in lipid-linked oligosaccharide synthesis in capillary endothelial cells by ~85% and the expression of cell surface N-glycans followed the cell cycle arrest in G1 and induction of apoptosis due to ER stress. High GRP-78/Bip suggested unfolded protein response. Up-regulation of c-Jun and c-Myc, and down regulation of c-Fos indicated the loss of survival signal. IGF-1 signaling down regulated phospho-Akt/GSK3beta/caspase-9/bad/mTOR and correlated with reduced ATP level. *In vivo*, tunicamycin inhibited $VEGF_{165}$-stimulated angiogenesis in Matrigel™ implants in nude mice. The expression of CD34 and CD144 was down regulated but that of thrombspondin-1 gene was increased in implants. Inhibition of $VEGF_{165}$-speciifc tyrosine kinase activity and a reduced phosphorylated VEGFR1 and VEGFR2 supported impaired VEGF signaling. Tunicamycin treatment of the breast tumor in nude mice exhibited a substantial reduction of the tumor growth (~50%-65%) in 1-4 weeks, and supported by reduced microvessel density, mitotic index, and Ki-67 and VEGF expression in the tumor tissue. The results strongly support tunicamycin's potential for a glycotherapeutic for treating breast cancer in the clinic. Supported in part by grants from Susan G. Komen for Cure BCTR06000532 (DKB) and NIH-NCRR G12-RR03035 (KB).

Program/Abstract# 177

Distinct roles of neural cell specific carbohydrates, polysialic acid and HNK-1 glycan, in cell-cell interaction and tumor formation

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Polysialic acid, a homopolymer of α2,8-linked sialic acid, and HNK-1, a sulfated and glucuronylated glycan, are involved in

neural cell migration, differentiation and plasticity. Polysialic acid and HNK-1 are co-expressed in some neurites, but are distinctively expressed in cultured neural cells and during brain development. Polysialic acid expressed in both neurons and substrate cells promote neurite outgrowth, while HNK-1 expressed on substrate cells but not on neurons promote neurite outgrowth. Although polysialic acid and HNK-1 epitopes are expressed on NCAM, cell aggregation assay showed inhibitory role of polysialic acid for NCAM-mediated cell-cell interaction, but not by HNK-1. Furthermore, polysialic acid but not HNK-1 glycan inhibits PDGF signaling through PDGF receptor (Mol. Cell. Biol., 27: 6659–6669, 2007) while both glycans reduce laminin-induced signaling, lowering MAP-kinase response. Importantly, the expression of polysialic acid is highly correlated with the progression of glioma in patients and increased migration of C6 glioma cells (Glycobiology, 15: 887–894, 2005). By contrast, the expression of HNK-1 is reversely correlated with the progression of glioma, and C6 cells expressing HNK-1 glycan produce a much smaller tumor when inoculated in the brain, most likely due to decreased cell migration. These findings demonstrate that polysialic acid and HNK-1 glycan exhibit opposing roles in tumor spreading and distinct roles in neural cell-cell interaction and cell-substratum interaction. Supported by CA338957.

Program/Abstract# 178

Glycosyltransferase gene expression manipulation as a therapeutic strategy for the treatment of malignant brain tumors

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Tumor cells display aberrant cell-surface glycosylation patterns brought about by alterations in their biosynthetic machinery. This holds true for highly invasive, malignant brain tumors as well as tumor cells that metastasize to the brain. We have found that glycosyltransferase gene transfection into human glioma cell lines has a marked impact on tumor cell growth, adhesion, invasivity, and increases in sensitivity to apoptotic-inducing chemotherapeutics in both *in vitro* and *in vivo* models. In particular we have found that clones of stable transfectants expressing the α2,6 sialyltransferase (α2,6ST) gene displayed marked inhibition of invasivity *in vitro* and formed no tumors in SCID mice intracranially implanted with these modified glioma tumor cells. Our results also showed alterations in integrin (specifically the β1 subunit glycosylation and alterations in focal adhesion kinase-associated signal transduction. Further a significant was observed. Recently, an adenoviral vector containing α2,6 ST (AdHAST) was constructed and shown to inhibit tumor formation *in vitro* and in preliminary studies in a panel of human tumor xenografts *in vivo* as well. These data underscore the opportunity to now answer questions as to how glyco-genes are regulated at the genomic and transcriptomic level which, in turn, could lead to development of novel therapeutics based on the direct regulation of tumor cell glyco-gene expression.

Program/Abstract# 179

Inhibition of U937-cell adhesion to human endothelial cells by glycosylated lysoyzme mutants

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In this study we expressed and characterized a glycosylated human lysoyzme mutant (hLysII/IVT-FUCTVI) that is able to inhibit cell adhesion of U937 cells to activated HUVEC-cells and could therefore represent a potential therapeutic option for treatment of inflammation and metastatic spread. Previously we have studied the elongation of oligosaccharides containing N-acetyllactosamine repeats and reported that a combination of glycosylation sites at the 49th (site IV) and 68th (site II) amino acid residues of the protein (hLysII/IVT) is efficiently stimulating the synthesis of N-acetyllactosamine repeats and that is the carbohydrate attached to site IV that is particularly affected. Now we transfected the cDNA of hLysII/IVT in CHO-FUCIII, IV, V and VI cells, characterized the glycosylation by glycosidase treatment and tested hLysII/IVT-FUCTIII-VI for their capability of inhibiting cell adhesion of U937-cells to activated human endothelial cells (HUVEC). Glycosidase treatment revealed that hLysII/IVT-FUCTVI and VII contain a terminal sLex-substance. In the adhesion assay we found a significant reduction of cell adhesion by hLysII/IVT-FUCTVI. The adhesion capacity (IC50: $7*10^{-12}$M) is the highest described to date and has to be confirmed in further (in vivo) assays.

Program/Abstract# 180

Role of N-acetylglucosaminyltransferase III and V in the post-translational modifications of E-cadherin

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E-cadherin dysfunction is a cause of epithelial cell invasion. Little is known about the Post-Translational Modifications of E-cadherin and its role in E-cadherin mediated tumor progression. N-acetylglucosaminyltransferase III (GnT-III) catalyzes the formation of a bisecting GlcNAc structure in N-glycans, and has been shown to act as a suppressor of metastasis. N-acetylglucosaminyltransferase V (GnT-V) catalyzes the addition of β 1,6 GlcNAc branching of N-glycans, and has been associated to increase metastasis. In the present study we have evaluated the regulatory mechanism between E-cadherin expression and the remodeling of its oligosaccharides structures by GnT-III and GnT-V. We demonstrated that wild-type E-cadherin regulates *MGAT3* gene transcription resulting in increased GnT-III expression. We also showed that GnT-III and GnT-V competitively modified E-cadherin N-glycans. The knockdown of GnT-III in cells resulted in a de-localization of E-cadherin to the cytoplasm together with a disruption of intercellular contacts. Further, the GnT-III knockdown cells also caused modifications of E-cadherin N-glycans catalyzed by GnT-III and GnT-V. Altogether our results demonstrated the existence of a bidirectional crosstalk between E-cadherin and GnT-III/GnT-V that was, for the first time, reproduced in an *in vivo* model. This study opens new insights into the post-translational modifications of E-cadherin in its biological function, in a tumor context.

Program/Abstract# 181

Carbohydrate microarrays toward deciphering the glycome

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Development of a detailed knowledge-base of biological systems that operate through saccharide recognition remains one of the most challenging areas of the biomedical sciences, but is now being greatly aided by carbohydrate microarray technology, in conjunction with recombinant protein expression for the study of carbohydrate-protein interactions. The neoglycolipid (NGL)- based oligosaccharide microarray system[1-4] now contains more than 600 unique sequence-defined probes encompassing diverse mammalian-type glycoprotein, glycolipid and glycosaminoglycan sequences (from natural sources and synthetic), also probes derived from bacterial, fungal and plant polysaccharides. Coupled with mass spectrometry it is already validated for ligand discovery by generating ‘designer’ microarrays from targeted macromolecules and tissue glycomes[5-9]. Customized software is in place for data interpretation, presentation, storage and mining. A special feature of the NGL-based microarray system is that the saccharide probes lend themselves well to live cell-based experiments to follow up the microarray binding data in relevant biological contexts. I will review recent highlights and consider prospects for biological systems that operate through saccharide recognition.

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Program/Abstract# 182

Cell signaling functions of C-mannosylated peptides from the thrombospondin type 1 repeat

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C-Mannosylation is unique in that an alpha-mannose is directly bound to the indole C2 carbon atom of a Trp residue through a C-C bond to produce C-mannosyl Trp (C-Man-Trp), and it occurs at the first Trp in the Trp-x-x-Trp motif, which is found in the thrombospondin type 1 repeat (TSR) of proteins. There are a number of examples of C-mannosylated proteins, such as thrombospondin, complements, Interleukin-12, and so forth. However, the biological function of protein C-mannosylation is not fully understood.

We previously reported that C-mannosylation is increased in specific tissues or cell types under hyperglycemic conditions, suggesting a pathological role of the increased C-mannosylation in development of diabetic complications (ref. 1). We also found that synthesized C-mannosylated TSR-derived peptides (*e.g.* C-Man-Trp-Ser-Pro-Trp) specifically enhanced LPS-induced signaling in macrophage-like RAW264.7 cells (ref. 2). We searched specific binding partners for C-mannosylated peptides in the cells, and identified heat shock cognate protein 70 (Hsc70) as a binding protein to the C-mannosylated peptides. The binding affinity of Hsc70 for C-mannosylated peptides was higher than that for the peptides without C-mannose. We will also demonstrate our results concerning the effect of C-mannosylated peptides on Hsc70-induced signaling in the cells, and discuss about possible functions of C-mannosylation in the cell signaling.

Supported in part by grants-in aid from the Ministry of Education, Culture, Sports, Science, and Technology of Japan, and the Mizutani Foundation for Glycoscience.

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Program/Abstract# 183

Modulation of cell functions by glycosphingolipid metabolic remodeling in the plasma membrane

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The endoplasmic reticulum and the Golgi apparatus are the subcellular sites for glycosphingolipid neobiosynthesis. In addition to this very well-established knowledge, it is now emerging that post-Golgi changes in glycosphingolipid structures occurring at the plasma membrane are an important opportunity to modulate cell glycosphingolipid composition and to affect consequently a number of signaling processes. In fact, it is possible to modify very rapidly the membrane organization by the modulation of plasma membrane-associated enzymes through external stimuli, thus affecting the membrane environment and the functional properties of plasma membrane proteins involved in cell signaling. The number of enzymes for glycosphingolipid metabolism that have been shown to be associated with the plasma membrane and the information on their features are growing very rapidly, and today some of these enzymes have been deeply characterized. In this review, we focus on the possible role and on the involvement of these plasma membrane-associated enzymes in modulating cell functions.

Program/Abstract# 184

Sialic acid biosynthesis is involved in proliferation and gene expression

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Sialic acids (Sia) represent a family of amino sugars with over 50 members, which are involved in a variety of biological interactions. The most prominent Sia in eukaryotes, and the precursor for all other Sia, is N-acetylneuraminic acid. N-acetylneuraminic acid is synthesized in the cytosol from UDP-N-acetylglucosamine by four reactions. The first two reactions are catalyzed by one bifunctional enzyme, the UDP-N-acetylglucosamine-2-epimerase/N-acetylmannosamine kinase (GNE). A central dogma of textbook knowledge is that GNE is the key enzyme of the sialic acid biosynthesis and therefore involved in the synthesis of sialoglycoconjugates. However, very recent studies suggest that GNE itself and probably also sialic acid itself plays an important role in cell regulation. Using GNE +/+ and GNE -/- embryonic stem cell lines, we here present data that strongly support this hypothesis. We show that GNE-expression (and consequently the sialic acid biosynthesis) is not only involved in the synthesis of sialoglycoconjugates, but also in proliferation, gene expression, and cell differentiation.

Program/Abstract# 185

Pathophysiological effects of anti-lipid A antibody on sodium channel activities: implication on Guillain-Barré syndrome

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Guillain-Barré syndrome (GBS) and its variants are autoimmune neuropathies that frequently occur as a result of an infectious event from agents such as *Campylobacter jejuni(C. jejuni)*. A strain of *C. jejuni*was confirmed in a GBS patient who had previously been exposed to chickens with campylobacteriosis. Sera from the affected chicken had anti-lipid A antibodies directed against the bacterial lipooligosaccharide (LOS). The anti-lipid A activities inhibited voltage-gated Na^+ currents ina motor neuron-like cell line, NSC-34, in culture. Treatment of the NSC-34 cells with tunicamycin had no effect on suppression of Na^+ currents, suggesting that the anti-lipid A induced a functional inhibition in the protein portion of the Nav channels. A rabbit polyclonal antibody (pAb) directed against a 19-mer peptide KELKDNHILNHVGLTDGPR on the α subunit of Nav1.4, present in NSC-34 cells, was generated and was found to cross-react with Kdo2-Lipid A. However, anti-Nav1.4 pAB itself did not inhibit the Na^+ currents in NSC-34 cells. Computational analysis revealed that the dipeptide (LK, Leu^3-Lys^4) near the N-terminus of the 19-mer peptide had a conformational similarity to the phosphorylated lipid A. The similarity was also predicted on the secondary structure of the extracellular loops in the α subunit of the Nav1.4 channel by molecular overlapping. We propose 12 epitopic dipeptides that provide potential binding sites for anti-Kdo2-Lipid A antibody. Binding of the anti-Kdo2-Lipid A antibody to one or more of these sites might lead to interference of Na channel property. Our results suggest that the serum anti-lipid A activity is involved in depressing the function of the Nav channel in whole-cell membrane currents. This inhibitory effect may contribute to neurophysiological changes in GBS by disrupting the normal function of the Na channels.

Program/Abstract# 186

O-Glycopeptide specific auto-antibodies for early detection of colorectal cancer

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Circulating antibodies from cancer patients hold promise as specific and sensitive biomarkers for the early detection of cancer. We have previously developed a microarray platform for screening of human antibodies to glycopeptides epitopes. Here we have produced a glycopeptide array presenting peptides derived from the human mucins MUC1, MUC2, MUC4, MUC5AC, MUC6, and MUC7 with different cancer-associated O-glycans. Using this mucin glycopeptide array we found that 86% of patients (48/56) with colorectal cancer have circulating antibodies against distinct glycopeptide epitopes in the tandem repeat regions of MUC1 and MUC4 carrying aberrant O-linked glycans including Tn (GalNAcα1-O-Ser/Thr), STn (NeuAcα2,6-GalNAcα1-O-Ser/Thr) and truncated core3 (GlcNAcβ1-3GalNAcα1-O-Ser/Thr). Controls included healthy blood donors and patients with chronic inflammatory bowel disease (IBD). Healthy donors and IBD patients showed no detectable antibodies to several of the glycopeptides epitopes for which cancer patients had antibodies (Tn and STn glycoforms of MUC1 and MUC4), while both groups less frequent (5% and 30%, respectively) had antibodies to a MUC1 glycopeptide epitope with truncated core3 O-glycans. The detected antibodies were glycopeptide specific as demonstrated by inhibition studies and discriminated between colorectal cancer patients and healthy individuals. Differential expression of the relevant target antigens were examined by two novel monoclonal antibodies raised against Tn-MUC4 and Core3-MUC1. We suggest that the use of glycopeptide arrays represents a promising diagnostic approach for early detection of cancer.

Program/Abstract# 187

Glycosylation of voltage-gated potassium channels affects neuronal expression and localization

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Potassium channels serve a variety of important physiological functions. In the nervous system, voltage-gated potassium (Kv) channels are important to control action potential waveform. Several Kv channels are glycosylated, though the function of native glycosylation has not been determined fully. We are studying the role of glycosylation in Kv1.2 channels using primary hippocampal neuronal cultures. The glycan structures of Kv1.2 channels were analyzed by glycosidase digestion, confirming different glycosylation in neuronal cultures compared to more commonly used cell culture lines. The importance of glycosylation for cell surface expression and localization of Kv1.2 was also analyzed, indicating a significance of native glycosylation for proper localization and function in neurons.

Program/Abstract# 188

Molecular mechanisms of O-GlcNAc signalling

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Many proteins in the eukaryotic cell are modified by O-linked N-acetylglucosamine (O-GlcNAc) on serines and threonines. O-GlcNAcylation has been shown to be important for regulation of the cell cycle, DNA transcription and translation, insulin sensitivity and protein degradation. Misregulation of O-GlcNAcylation is associated with diabetes and Alzheimer's disease. Two enzymes are involved in the dynamic cycling of this posttranslational modification, the O-GlcNAc transferase (OGT) and O-GlcNAcase (OGA). It has been demonstrated that this posttranslational modification occurs on some serines/threonines that are also known phosphorylation sites for a number of key kinases, giving rise to the "yin-yang" theory, that proposes that O-GlcNAcylation is a means of regulating protein phosphorylation. Work in my group is aimed at studing this mechanism. We are studying the structures of OGA and OGT to gain insight into substrate recognition, and we have developed highly potent andselective inhibitors to study O-GlcNAcylation in live cells. Using these tools were are currently studying the role of O-GlcNAc in signalling pathways involved in diabetes, cancer and Alzheimer's disease.

Program/Abstract# 189

Dynamic crosstalk between GlcNAcylation & phosphorylation: roles in signaling & human disease

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O-linked β-N-acetylglucosamine (O-GlcNAc) serves as a nutrient/stress sensor to modulate signaling, metabolism and gene expression (for review, *Nature*446, 1017–1022; *Am J Physiol Endocrinol Metab*295: E17-E28). O-GlcNAc plays a direct role in the etiology of diabetes, neurodegenerative disease, and cancer. Recent phospho-proteomic and glycomic studies have shown that the crosstalk between GlcNAcylation and phosphorylation is extensive at the individual site level. This dynamic interplay not only occurs by competition at the same or proximal sites, but also by each modification regulating the other's cycling enzymes. For example, several kinases are regulated by GlcNAcylation, and phosphorylation regulates both O-GlcNAc Transferase and O-GlcNAcase. The elucidation of this extensive crosstalk between these two most abundant protein modifications will have a major impact on our view of signaling and transcriptional regulation. Examples of the importance of this dynamic interplay in the regulation of FOXO transcription factors, kinases, RNA polymerase II, and cytokinesis will be presented.

Supported by NIH grants CA42486, DK61671, DK71280, and NIH contract N01-HV-28180. Dr. Hart receives a share of royalty received by the university on sales of the CTD 110.6 antibody. Terms of this arrangement are managed by JHUSOM.

Program/Abstract# 190

Hexosamine signaling: friend or foe in feast or famine

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Pathways of nutrient acquisition and salvage are evolutionarily linked to the cellular stress and immune systems. The cellular response to feast or famine is mediated by the concerted action of a variety of key signaling pathways including the AMP-kinase, mTOR and Hexosamine-signaling pathways. In turn, these pathways interact with, and serve to modulate, homeostatic mechanisms such as the Insulin signaling, TGF-ß and MAP kinase signaling cascades. The Hexosamine-signaling pathway is of particular interest since it is responsive to cellular levels of amino acids, sugars and ATP. Growing evidence suggests that O-GlcNAc, the end product of the hexosamine-signaling pathway, modulates intracellular signaling by its covalent attachment to key components of kinase-dependent signaling cascades. The enzymes of O-GlcNAc cycling are recruited to their sites of action by the same activation mechanism (PI-3 kinase) triggering Insulin and many other signaling cascades. Thus, the Hexosamine-signaling pathway impacts insulin signaling and other pathways by directly responding to nutrient availability. In *C. elegans, Drosophila,* and in mice, genetic evidence suggests that Hexosamine-signaling by O-GlcNAc serves as an epigenetic modulator of transcription, translation, and protein stability. O-GlcNAc is a mediator of Polycomb-group transcriptional repression and influences stem cell fate. The two key enzymes in this process, O-GlcNAc transferase and O-GlcNAcase have thus emerged as promising potential drug targets. The pathways impacted by the nutrient-responsive hexosamine-signaling pathway include the many of the key pathways (stress, immunity and metabolism) deregulated in metabolic syndrome. In fact, the O-GlcNAcase is a known diabetes susceptibility locus in Mexican Americans. A 'vicious cycle' exists in such populations; children of mothers with diabetes show increased risk for developing the disease due to unknown epigenetic factors in the intrauterine environment. Our current hypothesis is that O-GlcNAc cycling integrates metabolic information, potentially leading to epigenetic reprogramming in the intrauterine environment.

Program/Abstract# 191

Structure-function studies and design of novel Glycosyltransferases for site specific bioconjugation of mAbs and scFv via Glycan residues: development of a targeted drug delivery system and contrast agents for MRI

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The structural information on glycosyltransferases has revealed: (1) They have flexible loops which undergo conformational changes upon sugar-nucleotide binding and create the catalytic pocket and the acceptor binding site; (2) Metal ions often bind at the hinge region of the flexible loop; (3) N-acetyl groups are often embedded in a hydrophobic pocket; (4) There are "Add on domains" that impart acceptor specificities to a glycosyltransferase, and (5) The specificity of the sugar donor in these enzymes is determined by a few residues in the sugar-nucleotide binding pocket of the enzyme, conserved among the family members from different species. Mutation of these residues generates novel glycosyltransferases that can transfer a sugar residue with a chemically reactive functional group to N-acetylglucosamine (GlcNAc), galactose (Gal) and xylose residues of glycoproteins, glycolipids and proteoglycans

(glycoconjugates).The presence of a sugar moiety with a chemical handle on a glycoprotein makes it possible to conjugate site-specifically bioactive molecules with a corresponding orthogonal group via modified glycan moiety, thereby assisting in the assembly of bionanoparticles that are useful for developing the targeted drug delivery system and contrast agents for magnetic resonance imaging. The reengineered recombinant glycosyltransferases also make it possible to (1) remodel the oligosaccharide chains of glycoprotein drugs, and (2) synthesize oligosaccharides for vaccine development.

This project has been funded in whole or in part with federal funds from the NCI, National Institutes of Health, under contract N01-CO-12400.

Program/Abstract# 192

Oligosaccharide components of the Glycoprotein of the Opportunistic Pathogen *Scedosporium prolificans*

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The carbohydrate epitopes of glycoprotein of mycelium (RMP-Sp) from *Scedosporium prolificans* have been analyzed [1], and have been compared with those of its conidia (RMP-Sp-Coni). It contained 41% protein and Rha, Ara, Man, Gal, Glc, and $GlcNH_2$, plus 2MeRha (2%). The ^{13}C NMR spectra had similarities, but were not identical, and 2MeRha was a minor component (GLC-MS of alditol acetates), confirmed by ESI-MS of the mixture of oligos. formed on β-elimination [2]. This was applied to a Biogel P-2 column to give 16 fractions, from which successive F-3, F-4, F-9, F-12, and F-14 were chosen, based on their homogeneity (ESI-MS and HSQC NMR). F-4 was α-Rhap-(1→)-α-Rhap-(1→3)-α-Rhap-(1→2)-α-Manp-(1→2)-Man-ol, subst. at O-6 by β-Galp, since its DEPT spectrum was identical to a known specimen [1], whereas F-3 gave additional C-1, O-substituted, and -O$\underline{C}H_3$ signals. ESI-MS-MS (Li^+) showed that 2MeRha had replaced Rha (F-4) as a capping group. The only other fraction containing a 2MeRha capping group was as a trace in mixed F-7 (m/z 673; 2MeRha-Hex_2Hex-ol). Further analysis confirmed the structure of F-3. The DEPT [see (1)] of F-12, ESI-MS, and further analysis showed it to be β-Galp-(1→6)-[α-Manp-(1→2)]-Man-ol. F-14 contained mannitol (^{13})C NMR and ESI-MS).

[1] E. Barreto-Bergter *et al*. *Int. J. Biol. Macromolecules*, 42 (2008) 93–102.
[2] P.A.J. Gorin *et al*. *24th Int. Carbohydr. Symposium, Oslo* (2008) C-P057

Program/Abstract# 193

***In silico* and *in vitro* binding studies predict a novel sialic acid binding site on the sialic acid binding Ig-like lectin-7 (Siglec-7)**

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The Siglecs (Sialic acid Ig-like binding lectins) family is the largest group of the known vertebrate lectins recognizing sialylated glycans. Siglecs are involved in various cell-cell interactions and signaling events. Siglec-7 is an inhibitory receptor expressed on human natural killer cells, and shows dual specificity toward α2,8-disialylated glycans (Neu5Acα2-8Neu5Ac-) and α2,6-sialylated glycans (Galβ1-3[Neu5Acα2-6]HexNAc-). In this study, to understand the underlying mechanism for the dual specificity, *in silico* docking studies combined with biochemical binding assay were performed. Flexible docking algorithms, DOCK and eHiTS, were applied for analyzing the interaction between Siglec-7 V-set domain (Siglec-7 V) and various sialosides. The results showed: (i) All the sialosides examined predominantly interact with the primary docking site of Siglec-7 V, which was previously identified as a putative binding site by crystallographical and site-directed mutantagenesis analyses; (ii) In most models, α2,8-disialylated glycans interact with the conserved Arg124 in the primary docking site, its adjacent amino acids, and C-C' loop; (iii) The linkage type of Sia and core glycan structures affect the binding, consistent with the results of the ELISA-based binding experiments; (iv) In some models constructed by the docking simulations, α2,8-disialylated glycans also interact with a novel docking site other than the primary docking site. Interestingly, the novel docking site is prominently uncovered on the ligand-bound form in which a displacement of the C-C' loop of the apo form occurs. Furthermore, to examine if the novel docking site was actually involved in the binding with the ligands, we performed the site-directed mutagenesis and *in vitro* binding experiments of a Fc-conjugated Siglec-7 V expressed and purified from CHO cells. The binding of the α2,8-disialylated glycans with the Siglec-7-Fc was greatly affected by an R→A-mutation in the novel docking site.

Program/Abstract# 194

Novel aspects of the catalytic mechanism of human blood group B galactosyltransferase from NMR and microcalorimetry

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The human blood group B galactosyltransferase (GTB) catalyzes the transfer of galactose from uridine diphosphate galactose (UDP-Gal) to the C3 position of the terminal galactose of H-antigen acceptors. It is the last step in the formation of human blood group B antigens. The reaction proceeds with retention of configuration of the anomeric center of the donor sugar. Despite a number of studies the detailed mechanism for this class of enzymes remains unknown.In the absence of an acceptor substrate GTB catalyzes the hydrolysis of UDP-Gal into uridine diphosphate (UDP) and α-D-galactose - also with retention of configuration. We have explored the influence of acceptor-analog binding to GTB on the rate of hydrolysis of UDP-Gal by performing NMR experiments in conjunction with radioactive assays and isothermal titration calorimetry. The results show that the presence of acceptor-substrate analogs accelerates the enzymatic hydrolysis primarily by increasing the affinity for the donor substrate UDP-Gal. In consideration of recent crystallographic data [1] it is suggested that binding of acceptor-substrate to GTB in general leads to a conformational transition that accelerates glycosyltransfer.

[1] J.A. Alfaro, R.B. Zheng, M. Persson, J.A. Letts, R. Polakowski, Y. Bai, S.N. Borisova, N.O. Seto, T.L. Lowary, M.M. Palcic, S.V. Evans (2008) J. Biol. Chem. 283(15), 10097-108

Program/Abstract# 195

Ganglioside metabolism, the tumor microenvironment, and tumor progression

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To attempt to better understand the pathogenesis of human cancer, increasing attention has been directed to identifying potential in vivo interactions between the tumor cell and the surrounding tumor microenvironment–-the tumor-host interaction. Recent findings of our laboratory on the role of certain key membrane molecules, gangliosides, in influencing these tumor-host interactions will be reviewed. Briefly, by their synthesis and subsequent shedding from tumor cells, we hypothesize that tumor cell gangliosides enhance, and that interference with their synthesis impedes, tumor development and progression in vivo. Specifically, the characteristically rapid tumor cell ganglioside metabolism, *i.e.*, substantial synthesis resulting in substantial shedding into the tumor microenvironment, results in transfer to surrounding normal cells. Subsequently, these ganglioside-enriched normal cells have altered functions. Basic cellular mechanisms of these effects in the tumor microenvironment–-inhibition of the immune response, enhancement of stromal cell proliferation, and enhancement of the angiogenic response–-will be presented. To address a critical question raised by these studies, that of the effect on tumor formation itself, we sought to create a genetically stable, complete, and specific model of tumor cell ganglioside depletion. Preliminary findings on the selective and complete depletion of tumor cell gangliosides, in a murine sarcoma cell model, will be summarized, and the implications of these findings for future treatment of cancer considered.

Program/Abstract# 196

Therapeutic approaches for Tay-Sachs and Sandhoff disease models with recombinant human lysosomal β-hexosaminidase

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Tay-Sachs disease and Sandhoff disease are autosomal recessive GM2 gangliosidoses caused by lysosomal β-hexosaminidase (Hex, EC3.2.1.52) deficiency due to the primary defects of the human genes (*HEXA* and *HEXB*) encoding α- and β-subunit, respectively. These Hex deficiencies associate with excessive accumulation of GM2 ganglioside (GM2) in the brains and neurological symptoms. Among three Hex isozymes composed of α- and β-subunits, *i.e.* HexA (αβ), HexB (ββ) and a minor unstable HexS (αα), HexA is crucial for cleavage of the *N*-acetylgalactosamine residue from GM2 in co-orperation with GM2 activator protein. To develop enzyme replacement therapy (ERT) for Tay-Sachs disease and Sandhoff disease, we established a methylotrophic yeast *Ogataea minuta* strain (*Om4*) overexpressing the *MNN4* gene as well as *HEXA* and *HEXB*, and purified a novel recombinant human HexA carrying N-glycan with high content of

terminal mannose-6-phosphate (M6P) residues. Intracerebroventricular administration (*icv*) of the *Om4*HexA successfully restored the deficient enzyme activity due to the uptake via cation-independent M6P receptor (CI-M6PR) on neural cells, eliminated substrate storage in brain parenchyma and improved motor functions in a model mouse of Sandhoff disease as well as prolongation of lifespan, suggesting the therapeutic potential of *icv*ERT with the *Om4*HexA. We also designed *in silico* a human HexB with GM2-degrading activity based on comparison of the crystal structures between HexA and B. The purified gene products derived from a CHO cell line stably expressing the altered *HEXB* gene exhibited the catalytic activity for negatively charged artificial substrate, 4-methylumbelliferyl glucosaminide-6-sulfate, which were incorporated via CI-M6PR to correct the GM2 accumulated in the fibroblasts derived from a Tay-Sachs disease patient. This genetically-engineered human HexB should be also expected as a recombinant drug with low antigenecity applicable for intrathecal ERT for Tay-Sachs disease patients.

Program/Abstract# 197

Down regulation of galectin-3 expression in prostate adenocarcinoma is caused by its promoter hypermethylation: development and validation of a methylated marker for early diagnosis of prostate cancer

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Prostate cancer (PCa) is the second leading cause of cancer death in men. However, if PCa is diagnosed in its early stages such as stage I and II, it can be effectively treated and cured. But, unfortunately, the widely used PSA test is not suitable for early detection of PCa. Therefore, a reliable marker for early detection of prostate cancer is urgently needed. Genes, whose expression changes dramatically in the early stages of PCa, should serve as ideal markers in this regard. Galectin-3 (gal3), a member of the galactose-binding protein family, has been found to be poorly expressed in the early stages of PCa compared to the normal and benign prostatic hyperplasia (BPH) prostate tissues We have shown that the gal3 promoter in stage I and II PCa is heavily methylated and this hypermethylation may account for silencing of the gal3 gene in the early stages. Interestingly, the gal3 promoter in stage III and IV was lightly methylated. In normal and BPH prostate tissues, the gal3 promoter was unmethylated. Moreover, in PC-3 and DU-145 cells, gal3 is present at normal levels, whereas in LNCaP its expression is silenced. In LNCaP, the gal3 promoter was heavily methylated, whereas PC-3 or DU-145 cells showed negligible or no methylation in the gal3 promoter indicating a negative correlation between gal3 promoter methylation and its expression. Based on the cytosine methylation in the gal3 promoter in stages I-IV of PCa, we have developed sensitive and specific methylation-specific PCR assay that clearly identifies the early stages of prostate cancer in tissues as well as in biological fluids such as serum and urine (nearly 100% sensitive and nearly 100% specific). [Supported by UMBI Presidential Proof of Concept Award to H.A.]

Program/Abstract# 198

Translational aspects of Lactosylceramide Metabolism: mitigation of neovascularization and restensosis following balloon angioplasty

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Neo- vascularization leading to restenosis following ballon angioplasty in patients with coronary artery disease is a major problem with the current drug eluting stents. To examine the role of lactosylceramide synthase(LCS) and lactosylceramide(LC)in neo-vascularization, we have used the rabbit model of balloon angioplasty. Herein, rabbits were given intra-muscularly a daily injection of an inhibitor of LCS and GCS;D-PDMP , one day prior to surgery,and continued for 14 days. Hematoxylin-Eosin staining revealed that balloon angioplasty markedly induced neo-intimal proliferation compared to control rabbit. In contrast, D-PDMP completely reversed balloon angioplasty-induced neo-intimal proliferation. This was accompanied by a decrease in the mass of GC and LC as well as LCS activity. Furthermore, we have developed technology to load D-PDMP on to bio-degradable polymer-coated metallic stents and determined superb drug release kinetics. Such D-PDMP eluting stents do not cause bleeding or sepsis in rabbits. Our studies also show that D-PDMP –loaded on biopolymers do not interfere with VEGF-induced endothelial cell proliferation or induce apoptosis(measured by DAPI staining and cytochome c immunocytochemistry). Whereas , rapamycin/sirlolimus which targets mTOR did. In contrast, DPDMP-loaded on to biopolymer dose and time-dependently inhibited PDGF-induced proliferation of ASMC. In sum, we have developed D-PDMP eluting stents which may be efficacious and safe in increasing re-endothelialization and mitigating extensive neo-intimal proliferation in large animal models and in man.

Program/Abstract# 199

Modulating Glycosphingolipid synthesis for the treatment of type 2 diabetes and fatty liver disease

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Glycosphingolipids (GSLs) are major components of lipid rafts and have been shown to modulate the activity of numerous membrane-associated receptors, including the insulin receptor (IR). Studies in transgenic mice suggest a link between altered levels of various gangliosides and the development of insulin resistance. Here we show that an inhibitor of glycosphingolipid synthesis improved glucose control and increased insulin sensitivity in three different diabetic animal models. In the Zucker Diabetic Fatty (ZDF) rat, the glucosylceramide synthase inhibitor Genz-123346 lowered glucose and hemoglobin A1c (HbA1c) levels and improved glucose tolerance. Drug treatment also prevented the loss of pancreatic beta-cell function normally observed in the ZDF rat and preserved the ability of the animals to secrete insulin. In the Diet Induced Obese (DIO) mouse, treatment with Genz-123346 normalized HbA1c levels and improved glucose tolerance. Analysis of the phosphorylation state of the IR and downstream effectors showed increased insulin signaling in the muscles of both the treated ZDF rats and DIO mice. In the ob/ob mouse, besides lowering HbA1c levels, drug treatment markedly inhibited the development of hepatic steatosis, with significant reductions in the liver/body weight ratio, triglyceride levels, and several markers of liver pathology. These results suggest that inhibiting GSL synthesis can significantly improve insulin sensitivity, glucose homeostasis, and hepatic steatosis, and may therefore represent a novel therapeutic approach for the treatment of type 2 diabetes and fatty liver disease.

Program/Abstract# 200

O-GlcNAc modification on E-cadherin repressor snail confers Epithelial-Mesenchymal transition

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The transcriptional repressor Snail plays a key role in epithelial-mesenchymal transition (EMT) by which direct repression of E-cadherin transcription. Therefore, regulation of Snail expression level in epithelial tumor cells is important not only for maintaining of epithelial homeostasis, but also for invasion and metastasis of cancer cells by the EMT program. Series of Ser imbedded in Snail are phosphorylated by GSK3 and Snail expression is dynamically regulated by Wnt signaling together with β-catenin while driving a Snail-dependent EMT program. Glucose flux through the hexosamine biosynthetic pathway (HBP) can be used for the source of *O*-linked β-*N*-acetylglucosamine (*O*-GlcNAc) modification on Ser and Thr residues of various nucleocytoplasmic proteins. In this study, we demonstrate that Ser 112 of Snail is *O*-GlcNAcylated and this adjacent-site occupancy inhibits phosphorylation by GSK-3, resulting in increased Snail stability and attenuation of E-cadherin proximal promoter activity and transcription level. Furthermore, Overexpression of OGT induces *in vivo* invasion program of epithelial cancer cells by Snail-dependent manner. Taken together, our results indicate dynamic interplay between *O*-GlcNAcylation and GSK-3 phosphorylation of Snail, and our observations may provide the molecular insight of pathogenic and prognostic correlation between cancer progression and hyperglycemic condition of diabetes.

Program/Abstract# 201

Functional alteration of human β-hexosaminidase B for enzyme replacement therapy for GM2 gangliosidoses

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Tay-Sachs disease (TS) and Sandhoff disease (SD) are lysosonal storage disorders (LSDs) caused by a deficiency of lysosomal β-hexosaminidase (Hex, EC3.2.1.52) activity due to the primary defects of *HEXA* and *HEXB* encoding α- and β-subunit, respectively. These inborn errors resulting in excessive accumulation of GM2 ganglioside (GM2) in the brains and neurological manifestations.

There are three Hex isozymes composed of α- and β-subunits in mammals, HexA (αβ heterodimer), HexB (ββ homodimer) and a minor unstable HexS (αα homodimer). All these Hex isozymes can cleave off terminal *N*-acetylhexosaminyl residues of neutral oligosaccharides. Only HexA, however, is essential for cleavage of the *N*-acetylgalactosamine residue from GM2 in co-orperation

with GM2 activator protein (GM2AP) because α-subunit efficiently can bind negatively charged 6-sulfated hexosamine substrates and is association with GM2AP by α-specific loop structures.

To develop an enzyme replacement therapy for TS and SD, modified HexB (α-like HexB) with α-like substrate specificity and α-specific loop structures was designed on the bases of primary sequence comparison between α- and β-subunit. The human α-like HexB produced by CHO cell line acquired the activity of 4-methylumbellifery 6-sulfo-β-D-glucosaminide and is superior to the wild-type HexB in correcting the GM2 accumulated in fibroblasts from SD patients via cation-independent mannose-6-phosphate receptor (CI-M6PR). Furthermore, intracerebroventricular administration of α-like HexB successfully restored to the deficient Hex activity, eliminated substrate storage in thalamus and hypothalamus in a SD model mouse.

Program/Abstract# 202

Subtleties of substrate recognition by Calreticulin, a lectin chaperone

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Calreticulin (CRT) is a molecular chaperone of the endoplasmic reticulum (ER) which assists the folding of glycoproteins via interactions mediated by the glycan $Glc_1Man_{(5\text{-}9)}$ $GlcNAc_2$ present on the target glycoproteins. Previously, we have reported the thermodynamic parameters of its interaction with di-, tri-, and tetrasaccharides, the truncated versions of the glucosylated arm of $Glc_1Man_{(5\text{-}9)}$ $GlcNAc_2$, using the quantitative technique of isothermal titration calorimetry (ITC) (*Kapoor et. al, JBC 2003*). A 25 fold increase in binding constant was observed from di- to trisaccharide and 2 fold from tri- to tetrasaccharide, emphasizing that the entire Glcα1-3Manα>1-2α1-2ManαMe structure of the oligosaccharide is recognized by CRT. We have carried out modeling studies to identify the amino acids and hydroxyl groups of sugar moieties involved in hydrogen bonding. To support the observations and to determine the role of various hydroxyl groups of the substrate in sugar-CRT interactions we have used mono-deoxy analogues of the trisaccharide unit Glcα1-3Manα1-2ManMe. The failure of deoxy analogues to bind to CRT asserts the significance of the interactions between the primary binding site of CRT and the hydroxyl group of the concerned sugar residue in generating specificity for this recognition. Using thermodynamic data obtained by ITC with these analogues we validate that the 2-OH and 3-OH groups of Glc1 play an important role in sugar-CRT binding. Also, the 4-OH, 6-OH of Man2 and 3-OH, 4-OH of Man3 in the trisaccharide are involved in binding, of which 6-OH of Man2 and 4-OH of Man3 play a more significant role.

CRT shares high degree of similarity with Calnexin (CNX), another lectin chaperone in the ER. Modeling of CRT on CNX suggested that residues Asp-160 and Asp-317 are important for sugar binding. Using site-directed mutagenesis and ITC it was determined that the residue Asp-160 is not involved in sugar-binding, while Asp-317 plays a crucial role. Further, it was observed that cation-pi interactions with Trp-319 are involved in sugar-binding by CRT. Therefore, this study not only defines further the binding site of CRT but also highlights its subtle differences with that of CNX.

Program/Abstract# 203

Immobilization of unmodified glycans on the glass slide to construct carbohydrate microarrays

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Carbohydrate-protein recognition events play key roles in a variety of physiological and pathological processes. Carbohydrate microarrays have been extensively developed as reliable and efficient tools for the rapid analysis of glycan-protein interactions. The most general method for construction of these microarrays involves site-specific and covalent immobilization of chemically modified carbohydrates to properly derivatized surfaces. However, this immobilization strategy requires the use of properly functionalized sugars, which are typically prepared by multi-step synthetic sequences. In order to avoid the need for functionalized glycans, we have developed a direct, site-specific technique for immobilization of unmodified carbohydrates on the hydrazide-derivatized surface. To demonstrate the scope and applicability of this approach, carbohydrate microarrays containing fifty eight glycans have been constructed by using one-step, direct attachment of free carbohydrates to the hydrazide-coated surface. These microarrays have been employed for analysis of sugar binding specificities of lectins, antibodies and bacterial cells. In addition, this microarray format has been applied to the determination of binding affinities between proteins and glycans.

Program/Abstract# 204

Measurements of Glycosyltransferase activities using glycan arrays

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Carbohydrate microarrays have been used for the rapid analysis of glycan-protein, glycan-cell interactions and for

the detection of pathogens. A potentially important application of these microarrays is to assay glycosyltransferase activities. To date, only a few examples of enzymatic reactions taking place on the carbohydrate microarrays have been reported. However, this microarray technology has been rarely used in assaying glycosyltransferase activities. The approach requires only a tiny amount of immobilized acceptor substrates (picomoles), and enzymatic catalytic activities are readily assessed by measuring the amount of a product formed in a time-dependent manner. The level of time-dependent product conversion is determined by using fluorescence detection of lectin recognition of carbohydrate products.

Program/Abstract# 205

Superparamagnetic nanoparticles as platforms for studying protein-carbohydrate interactions

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It is known that complex oligosaccharides conjugated to the surface of mammalian cells via proteins or lipids are involved in the control of many normal and pathological processes. They influence differentiation, growth, adhesion and migration of cells. In turn, they are targets of many pathogenic viruses and bacteria in their initial infection cycles and also, the glycosilation pattern of cells changes due to certain diseases (hepatic diseases, cancer, etc) [1]. The study of these interactions is very challenging because to overcome their low affinity, it is necessary a multivalent presentation.

In this sense, Nps are good platforms to be used in these studies, as they stand in a similar size scale than biomolecules and allow a multivalent presentation of the ligands. We report the synthesis and characterization of glyco-magnetic nanoparticles (GMNps) with an excellent size control and high stability in physiological media. These GMNps were used to study carbohydrate-protein interactions which were monitored by changes in their aggregation state triggered by a target molecule, in this case a lectin (Concanavalin A). This aggregation induces changes in the relaxation time of water protons [2], which can be monitorized using a tabletop MR relaxometer. When Con A was added to a solution containing glucose-NPs, agglutination of NPs took place even at low concentrations of ConA (7 picomols). To show the specificity of these interactions, an excess of free glucose was added. As glucose would bind to ConA active sites, the clusters of GNPs disaggregated. Moreover, controls performed with galactose-NPs and heat inactivated ConA did not lead to any significant change in T_2 values, showing again the specificity of the interactions. These results show the great potential of these Nps as sensors for studying interactions where carbohydrates are implied.

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Program/Abstract# 206

Redesigning the carbohydrate recognition site of hen lysozyme

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In recent years, our research group has been involved in the study of protein-carbohydrate interactions, trying to clarify the underlying energetic-structural principles of the phenomenon. As a new stage in our investigation, we have aimed to redesign the protein's recognition site, changing its specificity towards other saccharidic structures. The primary target of the present project is to redesign the recognition site of hen's egg lysozyme, to make it specific for glucose β(1-4) oligomers. Using the crystal structure of lysozyme with chitotriose (GlcNAcβ(1-4)GlcNAcβ(1-4)GlcNAc) as a template, a model of lysozyme-binding cellotriose (Glcβ(1-4)Glcβ(1-4)Glc) was built. After several cycles of mutations and energy minimizations using Rosetta, a construct was obtained whose binding energy was comparable to that of the wild lysozyme-chitotriose complex. Mutations Ile98Gln, Ile58Gln and Leu75Arg optimized interactions with OH(2) groups in cellotriose. The optimal conformation of the two new Gln residues was stabilized by mutations Leu56Ser, Trp108Tyr, which make no direct contact with the ligand. Furthermore, mutations Ala107Asn and Trp63Tyr yielded increased affinity by improving interactions with common groups in cellotriose and chitotriose. Recombinant wild lysozyme was obtained in *Aspergillus niger*. Recombinant protein was characterized by means of circular dichroism and fluorescence spectroscopy, and its binding to chitotriose was measured calorimetrically. Overall, the secondary and tertiary structures, the thermal stability and the binding properties of recombinant lysozyme were identical to those of lysozyme purchased from SIGMA. Advances in the expression, purification and structural and energetic characterization of different lysozyme mutants are presented here.

Program/Abstract# 207

Nature of Lectin-carbohydrate interactions and how to treat them by computational chemistry tools

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Recognition in biological systems is often mediated through protein/carbohydrate interactions. Proteins that bind carbohydrates are called lectins. As the number of combinations that may create the saccharide code is extremely large, lectins must be very carefully designed by Nature to read and decipher such a complicated alphabet. The key role during the process of such deciphering is played by protein/carbohydrate interaction energy.

The forces that keep lectin/carbohydrate complexes together are based either on polar interactions between the polar carbohydrate groups, represented especially by oxygen atoms, and polar protein functional groups. This kind of interactions may often be mediated or enlarged by bridging water molecules, or by ions. The last option usually generates strong interactions leading to stable lectin/carbohydrate complexes. However, there are lectins that do not have enough polar amino-acid residues in the binding site but are still able to create stable enough complexes with carbohydrates. In this case, polar interactions are usually substituted by stacking interactions similar to what is observed in nucleic acids.

We will show in this paper how the above described different kind of complexes can be treated by computational methods. It will be demonstrated by some docking experiments on selected lectins from pathogens.

Program/Abstract# 208

Glycoconjugates (GC) in discrimination of GC-recognition systems of probiotic microorganisms. New potential keys for strains and their glycometabolome typing

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Introduction. GC recognition is ancient conservative functional property of microorganisms, the basis for relationships in survival communities. There are systems (S) of microbial lectins (L), L-like proteins/protein complexes/ensembles involving in sensing of GC. Microbial LS are potential initiators, organizers and regulators of the surrounding glycometabolome networks in cenosis (mixed microbial culture, human superorganism). Galactoside and mannoside recognition LS were found in cultural media of human intestine probiotic dacterial strains producing mainly acid protein massive [1]. **Method [1]**. We used selected panel of GC (www.lectinity.com) as analogs of human cell antigens (Tn, Adi, Forssman, others), intestinal mucins, carbohydrate moieties of glycoproteins, bacterial peptidoglycans, rhamnan, yeast mannans, alga fucan. The main tested probiotic strains were lactobacilli (3 species) and bifidobacteria (7 species). **Results**. A number of GC detected different LS that varied in pI sets. These LS were characterized by multiple forms distribution pictures that were similar within genera, species or strains. LS revealed could be classified in hierarchy order (more extended/narrow in acid pI sets as potentially more ancient (less specific)/ less ancient(more specific) respectively). They included similar (overlapping) and different (additional specific) L forms. **Conclusion**. Results demonstrate evolutionary selected mosaic type of GC discrimination by probiotic bacteria. The data point out the usefulness of selective GC as well as LS revealed for choice of probiotic strain type needed. It seems, such GC and LS found can be used for strains and their glycometabolomed typing. LS described may have industrial, biotechnological and medical perspectives.

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Program/Abstract# 209

Carbohydrate-sensitive proteins in study of glycoenzymes

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The data of the use of a extended panel of different lectins and enzymes (with known given specificities)in isolation/separation (in semipreparative scales), immobilization, and investigation of approx. 450 enzymes (ordred according to EC classes) are summerized as electronic glycobook in English. The data include detailed information and subreview comments on 57 oxydoreductases (EC), 72 transferases, 63 esterases, 68 glycosidases, 127 proteases, other hydrolases,lyases, isomerases and ligases.The book also includes enzyme sources (>400 species *in Latin,* cell types, lines or strains, biological fluids, tissue or organ types, extra- or intracellular, organelle type), sequential sorbents used (most effective step in isolation/separation as marked), other treatments/procedures, multiple glycoforms, glycan moiety that important for enzyme activity. Disease specific mammalian glycoenzyme forms, microbial enzymes of industrial importance, recombinant glycoforms, glycoprotein modulators of enzymes are also pointed out.

About 2 thousands of selected references are given. The content of glycobook also reflects the historical views of investigators that are of importance for modern scientists. The book can serve as introduction to some aspects of glycometabolomics and glycomics. Such a book is needed for post-graduate students, beginners and specialists in the fields of applied glycoenzymology, glycobiotechnology, glycobiochemistry, glyco(micro)biology, glycomedicine, human tumor diseases.

Program/Abstract# 210

High-specificity affinity reagents for *N*-glycosylation site mapping and glycomics

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To effectively employ and discover glycan disease markers, new glycan-specific reagents are urgently needed. Using structurally-guided genetic manipulations, we are converting oligosaccharide-processing enzymes into high affinity oligosaccharide-binding reagents. Here we report on our progress to convert the peptide: *N*-glycanase F (PNGase F) enzyme into a high-specificity affinity reagent for peptides that contain asparagine-linked carbohydrate chains. Because such a protein has lectin-like properties, but is derived from an enzyme, we are calling them "lectenz". The PNGase F lectenz may directly address the needs of glycomics/proteomics analysis through sample enrichment, thus facilitating glycosylation site-mapping. The initial PNGase F lectenz scaffold was generated from the enzyme produced by *Flavobacterium meningosepticum*, for which a crystal structure has been reported in complex with the disaccharide core common to *N*-linked glycans[1]. As first steps to developing a lectenz specific for *N*-linked glycans for proteomic and glycomic applications, we have 1) used site-directed mutagenesis to create a catalytically inactive version of PNGase F, 2) measured the binding of this immobilized *N*-glycan lectenz to a glycoprotein by surface plasmon resonance (SPR), and 3) used computational structural modeling to identify amino acid residues of PNGase F that play important roles in *N*-glycan binding specificity and affinity.

Reference:

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Program/Abstract# 211

Combining computational carbohydrate threading with glycan array data to define the 3D Epitope of an anti-tumor antibody

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Glycan array screening is a high throughput method for identifying potential binding partners for carbohydrate receptor proteins. However, such screening provides no insight into the origin of the observed binding or lack of binding among related glycans. When applicable, crystallographic and NMR methods can provide 3D structures for these complexes, however these methods are neither rapid nor high throughput. Automated carbohydrate docking offers one approach to generating putative models for these complexes, the accuracy of which may be greatly enhanced by selecting theoretical models that satisfy all of the observed binding and non-binding interactions identified by experimental screening. Once a minimal binding motif is identified experimentally, this motif is computationally docked to the protein receptor. The optimal theoretical alignment is identified from the numerous docked poses by superimposing (threading) the intact glycan, identified from experimental screening, through each motif pose. By considering both binding and non-binding glycans that contain the minimal motif, it is possible to unambiguously identify the optimal orientation of the ligand in the binding site, providing not only a 3D model for the complex, but also a rational explanation for the observed array data. We illustrate this high-throughput approach for the anti-tumor antibody JAA-F11, which recognizes the Thomsen-Friedenreich (TF) alpha-linked antigen. Using recently reported data from screening against the glycan array at the Consortium for Functional Glycomics and a crystal structure of the free protein, we derive a 3D structure for the antigen-antibody complex.

Program/Abstract# 212

Glycan receptor-binding specificity in human adaptation of influenza a virus Hemagglutinin

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The binding specificity of the influenza A virus hemagglutinin (HA) to sialylated glycan receptors on the host cell surface plays a critical role in the viral adaptation to that host. This binding specificity has been broadly defined based on the a2-3 or a2-6 linkage of the terminal Neu5Ac sugar to the penultimate Galactose sugar in complex cell surface glycans. However, this definition has led to confounding issues in understanding human adaptation of the HA especially in relation to the airborne transmissibility of the viruses. Our approach towards addressing these issues has been to integrate a complementary set of tools to understand glycan receptor binding specificity of HA by incorporating structural diversity in the glycan receptors and multivalency in the HA-glycan interactions. Using such a multifaceted approach we have shown that glycan topology and quantitative glycan binding affinity are important parameters that determine human adaptation of HA for efficient human to human viral transmission. Recently we have extended our approach to correlate the glycan-binding specificity of the 2009 H1N1 'swine flu' HA with the transmissibility of this virus.

Program/Abstract# 213

ST3Gal.I sialyltransferase relevance in bladder cancer tissues and cell lines

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Almost every type of cancer expresses deranged glycosidic structures, an event expected to involve the altered expression of sialyltransferases (ST). Particularly, in bladder cancer the relevance of STs is not elucidated. The T antigen is a tumor-associated structure whose sialylation has been related with worse prognosis. In this study, we attempted to study the role of the STs able to sialylate the T antigen in bladder cancer progression. Patient matched pairs of bladder tumours and normal urothelium samples, and four bladder cancer cell lines were screened for: *ST3Gal.I*, *ST3Gal.II* and *ST3Gal.IV* mRNA level by real-time PCR; sialyl T antigens expression by dot blot and flow cytometry using PNA lectin and the two *ST3Gal.I* transcript variants. ST activity against the T antigen was analyzed in the cell lines.

In nonmuscle-invasive bladder cancer, *ST3Gal.I* mRNA levels were significantly higher than urothelium ($p<0,001$) and the increase appears to be twice more pronounced in cancers with tendency for recurrence. In muscle-invasive bladder cancer, elevation of *ST3Gal.I* mRNA levels was observed in both tumors and urothelium. Cell line analysis revealed a good correlation between the *ST3Gal.I* mRNA level and ST activity towards the T antigen ($r=0{,}99$; $p=0{,}001$) and these results were in agreement with the cell sialyl T antigen expression. Patient bladder tumors and urothelium also expressed the sialyl T antigens but we were not able to directly associate this phenotype with the *ST3Gal.I* mRNA levels (nor ST3Gal.I and ST3Gal.IV). In most of the samples, the two *ST3Gal.I* transcript variants were equally expressed, without an evident correlation with cell phenotype or malignancy. Our results suggest that increased ST expression, in particular ST3Gal.I, is part of the oncogenic transformation of bladder. ST3Gal.I contributes to the sialylation of T antigens in bladder cancer and its RNAm levels can be considered for progression and recurrence prognosis. Presently we are establishing experimental models to assess the role of this ST in bladder cancer.

Program/Abstract# 214

N-Glycosylation of eFactor VIIIc is reguired for endothelial cell proliferation and Matrigel™ invasion

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Factor VIIIc is a large regulatory cofactor glycoprotein in the blood coagulation cascade. It has 6 sequential domains arranged in the order of A1-A2-B-A3-C1-C2. The circulating factor VIII protein however is a metal bridged heterodimer consisting of a heavy chain (A1-A2-B) and a light chain (A3-C1-C2). We have shown earlier the expression of Factor

VIIIc-like glycoprotein in capillary endothelial cells (*i.e.*, eFactor VIIIc) with a biological activity of that of Factor VIIIc. The secretory eFactorVIIIc is a M_r 270,000 dalton asparagine-linked (N-linked) glycoprotein in which the heavy chain M_r 210,000 dalton and the light chain M_r 46,000 dalton are joined together by disulfide-bridge(s). eFactorVIIIc is perinuclear and its expression precedes the capillary endothelial cell proliferation. The objective of our study has been to understand the role of N-glycans on eFactor VIIIc in angiogenesis. We have observed that endothelial cells cultured in the presence of anti-Factor VIIIc monoclonal antibody fail to invade the Matrigel™ matrix. To test the role of eFactorVIIIc N-glycans on cellular proliferation and tissue invasion cells are cultured in the presence of a protein N-glycosylation inhibitor, tunicamycin and the results support that (i) inhibition of eFactor VIIIc N-glycosylation with tunicamycin inhibits angiogenesis; and (ii) tunicamycin treatment exhibits considerable inhibition of Matrigel™ invasion. We, therefore, conclude that eFactor VIIIc is involved in matrix dissolution during tumor invasion of blood vessels by activating the matrix metalloproteinases (MMPs). Supported in part by grants G12-RR03035 (KB) and the Susan G. Komen for Cure BCTR0600582 (DKB).

Program/Abstract# 215

The role of anti-gal antibodies in the development and progression of pre-malignant liver disease

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Conventionally, antibodies can control an infection through neutralization or through the destruction of target cells. Paradoxically, we have determined that patients with liver fibrosis, regardless of the etiology of disease, have antibodies to bacteria that are altered both in amount, and in glycosylation, that do not control bacterial growth but actually appear to promote it. These antibodies, called anti-gal antibodies, are immunoglobulin molecules reactive towards the alpha-gal epitope, a carbohydrate found on many gram-negative bacteria. As bacterial infection is a major complication in patients with cirrhosis and bacterial products such as LPS are thought to play a major role in the development and progression of liver fibrosis, this finding has many clinical implications in the etiology, prognosis and treatment of liver disease. The change in glycosylation on anti-gal IgG allowed for increased reactivity with several fucose-binding lectins and permitted the development of a plate based assay to measure this change. Increased lectin reactivity was observed in 100% of the more than 200 individuals with stage III or greater fibrosis and appeared to correlate with the degree of fibrosis. This alteration in the glycosylation of anti-gal IgG is related to the development of a humoral immune response against bacterial products. Evidence is presented that this antibody plays a role in the exposure of the liver to bacterial products such as lipopolysaccharide (LPS) and may be involved in the development and progression of liver fibrosis.

Program/Abstract# 216

Role of E-cadherin N-glycosylation profile in a mammary tumor model

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Modifications in cell surface glycosylation affecting cell adhesion are common characteristics of transformed cells. This study characterizes the N-glycosylation profile of E-cadherin in models of canine mammary gland adenoma and carcinoma evaluating the importance of these glycosylation modifications in the malignant phenotype. Our results show that the pattern of E-cadherin N-glycosylation in mammary carcinoma is characterized by highly branched N-glycans, increase in sialylation and an expression of few high mannose structures. Three putative sites of N-glycosylation, shared by human, mouse and canine have been described in the extracellular domain of E-cadherin. Detailed mass spectrometry analysis demonstrated, for the first time, that canine E-cadherin can have a fourth site (Asn-775) that is N-glycosylated most likely with a complex type N-glycan, possibly a β1,6 branched GlcNAc form, located on the fifth extracellular domain (EC5) of E-cadherin. This site is also present in mouse but not in human E-cadherin. These results support that changes in glycosylation play crucial roles in the process of malignancy of mammary gland and in regulation and modification of E-cadherin-associated cell–cell adhesion which could lead to the malignant behaviour of tumor cells.

Program/Abstract# 217

Up-regulation of plasma membrane-associated sialidase augments malignant properties of cancer cells through activation of EGFR signaling

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Aberrant sialylation is a characteristic feature of cancer cells associated with malignant properties. To elucidate the causes and the consequences, our studies have been focused on human sialidase, which catalyzes removal of sialic acid residues from glycoproteins and glycolipids. We previously demonstrated that plasma membrane-associated sialidase (NEU3) is markedly up-regulated in various human cancers (1). NEU3 has been implicated to play important roles in regulation of cell signaling by ganglioside modulation, because of its strict substrate preference. Up-regulation of NEU3 increases invasiveness and motility and suppresses apoptosis of cancer cells, probably through activation of EGFR signaling pathway (3). In the present study, we found that NEU3 regulates EGFR signaling through interaction with the receptor as well as through ganglioside modulation. NEU3 up-regulation enhanced tyrosine-phosphorylation of EGFR the most at Tyr-845 and consequently activation of Ras cascades, especially via Ras/ERK pathway. On the other hand, NEU3 silencing resulted in inhibition of Ras cascades. To elucidate the molecular mechanism of NEU3-mediated activation of EGFR signaling, we observed whether the specific binding domains exist in NEU3 and EGFR. Transfection of expression vectors for EGFR and NEU3 fragments revealed that NEU3 is likely to associate with tyrosine kinase domain of EGFR. NEU3 effects on EGFR mutations, which are often observed in cancer patients, are now under investigation. NEU3 could be a potential target for cancer diagnosis and therapy.

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Program/Abstract# 218

Suppression of FUT4 down-regulates the expression and activation of PKCa and increases apoptosis of A431 cells

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LeY is a difucosylated oligosaccharide with the chemical structure [Fucα1→2Galβ1→4(Fucα1→3)GlcNAcβ1→R]. It is abnormally expressed in the majority of carcinomas derived from epithelial tissues. The α-1,3 fucosylation of LeY is catalyzed by fucosyltransferase IV (FUT4) which is a critical enzyme that controls LeY oligosaccharide synthesis. The previous work in our lab showed that decreasing the LeY level by FUT4 RNAi transfection or blocking functional LeY with specific LeY antibody incubation inhibited the A431 cell proliferation, whereas overexpression of FUT4 increased the synthesis of LeY and inhibit cell apoptosis. It is found that PKCα is closely related to the growth and metastasis of cancers, and is applied as a potential anti-cancer drug in clinic trial. To further explore the effect of FUT4 on PKCα expression and activation, as well as the cell proliferation and apoptosis, we used FUT4 RNAi sequence and PKCα antisense oligonucleotides to transiently transfect the A431 cells cells, and investigate the cell growth and apoptosis. The results showed that transfection with FUT4 RNAi sequence inhibited the expression and activation of PKCα compared with the control and vehicle by Western blot, and induced the cell apoptosis by Hoechst 33324 and AnnexinV/PI staining, as well as cell cycle analysis. The expression of PKCα was decreased when the cells were transfected with PKCα antisense oligonucleotides by Western blot. Cotransfection of FUT4 RNAi sequence and PKCα antisense oligonucleotides significantly increased the cell apoptosis compared with the control, vehicles, transfection with FUT4 RNAi sequence or PKCα antisense oligonucleotides. The results suggest that the expression and activation of PKCα was associated with FUT4. FUT4 may induce the apoptosis of A431 cells through inhibiting the expression and activation of PKCα.

This work was supported Specialized Research Fund for the Doctoral Program of Higher Education of China (20060161001) and Fund for Creative Research Groups of Liaoning Provice (2007 T021).

Program/Abstract# 219

Cytoplasmic expression of GlcA-GlcNH$_3^+$ on heparan sulfate closely correlates with malignancy of breast cancer

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Expressions of heparan sulfate glycosaminoglycans (HSGAGs) in totally 60 cases of breast cancer specimens were immunohistochemically investigated using monoclonal antibodies that recognize different epitopes of the glycan structures. Cytoplasmic expression of unordinary structure of GlcA-$GlcNH_3^+$ on HSGAG was characteristically detected in invasive carcinomas with high frequency, whereas hardly detected not only in non-invasive carcinomas but also in normal breast ducts. The expression-score of GlcA-$GlcNH_3^+$ significantly correlated with nuclear atypia score (p=0.0004), mitotic counts score (p=0.0018), nuclear grade (p=0.0061), and incidence of metastasis to axillary lymph nodes (p=0.0061). The score inversely correlated with expression of hormone receptors of estrogen (p=0.0028) and progesterone (p=0.0211). Interestingly the GlcA-$GlcNH_3^+$ was limitedly expressed in the cytoplasm of normal epithelial cells located in proliferative compartment of small intestine. These results indicate that a specific structure of GlcA-$GlcNH_3^+$ on HSGAG expressed in cytoplasm may concern with cell proliferation activity, thereby correlating with high malignancy. The expression GlcA-GlcNH3+ recognized by JM403 can be expected to be a novel biomarker to detect high malignant cells in breast cancers.

Program/Abstract# 220

Imaging technology of sialo-glycoconjugate molecular species by a combination of TLC-Blot and MALDI-TOF

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Nowadays, mass spectrometric (MS) analysis of glycol-chains shows a tremendous progress by the evolutional development of MS instrumental technology with the availability of glycol-chain library. We have been engaged in the characterization and functional elucidation of glycosphingolipids (GSLs). In the case of GSLs, in addition to the complexity of glycol-chains, variety of molecular species of hydrophobic moiety is quite abundant. The functional significance of the hydrophobic moiety and combination with glycol-chains should be clarified in terms of cellular behavior and organization. We have developed the transfer system of separated GSLs on HPTLC plate to PVDF membrane (TLC-Blot, named Far-Eastern Blot), and direct MS analysis of GSLs on the membrane. In the present study, we have developed molecular imaging technology of GSLs using gangliosides obtained from human brain samples. All the lipids blotted on a PVDF membrane from HPTLC plate were analyzed by MALDI-TOF MS, and the imaging of molecular spaces of individual ganglioside was obtained by scanning the blotted ganglioside bands. Distribution of molecular species has been clearly demonstrated as an image of hydrophobic variety of the ganglioside. In the course of this study, we developed an efficient blotting method for sialo-glycoconjugates. We will show the imaging profiles of different ganglioside molecular species from human hippocampus and inferior frontal gyrus. This method provides not only visual information but also a light to find possible functional significances of the combination of glyco-chain and hydrophobic moiety of sialo-glycoconjugates.

Program/Abstract# 221

Chemical characterization of oligosaccharides in chimpanzee, bonobo, gorilla, orangutan, and siamang milk or colostrum

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Neutral and acidic oligosaccharides were isolated from the milk or colostrum of four great ape species (Chimpanzee (*Pan troglodytes*), bonobo (*Pan paniscus*), gorilla (*Gorilla gorilla*), and orangutan (*Pongo pygmaeus*) and one lesser ape species (siamang (*Symphalangus syndactylus*)), and their chemical structures were characterized by 1H-NMR spectroscopy. Oligosaccharides containing the type II unit (Gal(β1-4)GlcNAc) were found exclusively (gorilla and siamang) or predominantly (chimpanzee, bonobo, and orangutan) over those containing the type I unit (Gal(β1-3)GlcNAc). In comparison, type I oligosaccharides predominate over type II oligosaccharides in human milk, whereas nonprimate milk almost always contains only type II oligosaccharides. The milk or colostrum of the great apes contained oligosaccharides bearing both N-glycolylneuraminic acid and N-acetylneuraminic acid, whereas human milk contains only the latter.

Reference: T. Urashima *et al*., Glycobiology 19, 499-508, 2009.

Program/Abstract# 222

Molecular mechanism for differential recognition of the C-5 substituents of sialic acid by CMP-sialic acid synthetases: identification of the evolutionally conserved sialic acid recognition region

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The activation of sialic acids (Sia) to CMP-Sia is a prerequisite for the *de novo* synthesis of sialoglycoconjugates. A CMP-Sia synthetase (CSS) catalyzes the synthesis of CMP-Sia from CTP and Sia. One of the interesting features of CSS resides in the differential recognition of Sia depending on species. The catalytic domain of CSSs in vertebrates exists in N-terminal half of the enzyme and there are five conserved amino acid sequence motifs (motifs I-V). The mouse CSS exhibits a preferential specificity for N-acetylneuraminic acid (Neu5Ac) to deaminoneuraminic acid (KDN), while the rainbow trout CSS is equally active with either of these Sia species. To identify structural elements for recognition of the Sia species, we analyzed the chimeric and site-directed mutants of the mouse and rainbow trout CSSs for the in vitro activity toward Neu5Ac and KDN using the sialic acid lyase/lactate dehydrogenase assay [Fujita *et al.* (2005) Anal. Biochem. 337, 12]. We showed that a limited amino acid sequence at the C-terminal region of the catalytic domain was involved in recognition of Sia species. To confirm the importance of elements, we further analyzed several other CSSs from fishes and prochordates for the structure of the Sia recognition region as well as the enzyme activity. We also predicted the steric structure of the region by molecular modeling. These results suggest that the orientation of a side chain of a particular Leu residue toward the C-5 substituent of Sia determines the Sia recognition specificity. This study was supported in part by JST CREST and JST SICP.

Program/Abstract# 223

Insights into evolutionary history of animal sialyltransferases

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The animal sialyltransferases catalyzing the transfer of sialic acid to the glycan of glycoconjugates comprise four families (ST3Gal, ST6Gal, ST6GalNAc and ST8Sia) that share four conserved peptide motifs (L, S, III and VS). These sialylmotifs are hallmarks for the identification of sialyltransferases. Twenty sialyltransferase gene subfamilies have been described in higher vertebrates and examples of the four families were also found in invertebrates. Focusing on the ST8Sia family, which ensure poly-, oligo- and mono-α2,8-sialylation, we investigated the origin of three groups of α2,8-sialyltransferases described in vertebrates. In the genome of deuterostomes, we identified orthologs for each of these three ST8Sia groups and a set of novel genes named *ST8SiaEX*, not found in vertebrates, which share a new conserved ST8Sia family-motif. We got insights into the duplications events that led to ST8Sia diversification through paralogons and synteny analysis and we proposed a scenario of the evolution of ST8Sias: Their main divergent functions are acquired from a series of tandem duplications and translocation long before vertebrate emergence, whereas other genes are secondary to whole genome duplications. However, many of the duplicated genes have been lost during animal evolution. Focusing on the ST6Gal family, we confirmed the occurrence of a unique ancestral *ST6Gal* gene very early in the Metazoan lineage. Two subsets of ST6Gal genes (*ST6Gal1* and *ST6Gal2*) arose from the second round of whole genome duplication (WGDR2) about 450 million years ago in early vertebrates. Our synteny studies and gene organization analysis pointed out a disruption in the conservation of the synteny of *ST6Gal1* gene in fish genomes suggesting a translocation event of this newly duplicated gene very early after teleost divergence.

Program/Abstract# 224

Inhibition of ganglioside biosynthesis as a novel therapeutic approach in insulin resistance

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Caveolae are a subset of membrane microdomains (lipid raft) particularly abundant in adipocytes. Critical dependence of the insulin metabolic signal transduction on caveolae/microdomains in adipocytes has been demonstrated. These microdomains can be biochemically isolated with

their detergent insolubility and were designated as detergent resistant microdomains (DRMs). Gangliosides are known as structurally and functionally important components in microdomains. We demonstrated that increased GM3 expression was accompanied in the state of insulin resistance in mouse 3 T3-L1 adipocytes induced by TNFalpha and in the adipose tissues of obese/diabetic rodent models such as Zucker *fa/fa* rats and *ob/ob* mice[(1)]. We examined the effect of TNF on the composition and function of DRMs in adipocytes and demonstrated that increased GM3 levels result in the elimination of insulin receptor (IR) from the DRM while caveolin and flotillin remain in the DRMs, leading to the inhibition of insulin's metabolic signaling. These findings are further supported by the report that mice lacking GM3 synthase exhibit enhanced insulin signaling[(3)]. To gain insight into molecular mechanisms behind interactions of IR, caveolin-1 (Cav1) and GM3 in adipocytes, we have performed immunoprecipitations, cross-linking studies of IR and GM3, and live cell studies using fluorescence recovery after photobleaching (FRAP) technique. We found that (*i*) IR form complexes with Cav1 and GM3 independently; (*ii*) in GM3-enriched membranes the mobility of IR is increased by dissociation of the IR-Cav1 interaction; (*iii*) the lysine residue localized just above the transmembrane domain of the IR β-subunit is essential for the interaction of IR with GM3. These evidence substantiate that insulin resistance in adipocytes is caused by dissociation of the IR–Cav1 complex by the interactions of IR with GM3 in microdomains[4]. In addition, our data substantiate a novel diagnostic strategy for metabolic disorders by measuring the circulating levels of GM3[(5)].

In this talk, I will demonstrate a new concept "Metabolic diseases, such as type 2 diabetes, are a membrane microdomain disorder caused by aberrant expression of gangliosides" and propose the new therapeutic strategy "membrane microdomain ortho-signaling therapy".

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Program/Abstract# 225

Link between quality control and ERAD process of newly synthsized glycoproteins

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In mammalian cells, the endoplasmic reticulum is the major site for membrane and secretory N-glycoproteins biosynthesis. Before sending to their final site of functions, newly synthesized glycoproteins must get over the so-called ER quality control. Consisting of lectins and enzymes such as calnexin, calreticulin, UGGT, this system, exclusively indorsing on the glycan structure, tends to properly fold the newly synthesized glycoproteins that are unfolded. Despite this rigorous machinery about 20% of glycoproteins remains misfolded and are thus engaged into ERAD (endoplasmic reticulum-associated degradation) pathway. Intriguingly, the glycan structure governs the fate of the glycoproteins.

While protein backbone is exclusively degraded in the cytosol, released free oligosaccharides are catabolized both in the cytosol and lysosomes by a set of enzymes. The study of free oligosaccharide structures allows the understanding of the molecular mechanisms of quality control and ERAD. Even if the sequential events of ER quality control and ERAD are well described, their regulations remain poorly understood. Recently, we demonstrated that MAN2C1 overexpression, the alpha-mannosidase involved in the catabolism of cytosolic free oligosaccharides would increase the ERAD rate correlated to an accumulation of small free oligosaccharides in the cytosol. These results would offer new insights about the importance of cytosolic free oligosaccharides in glycoprotein metabolism.

Program/Abstract# 226

Towards complete molecular definition of the cell wall of *Mycobacterium tuberculosis* and *Mycobacterium leprae*

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Much of the early structural definition of the cell wall of *Mycobacterium* spp. was initiated in the 1960 s and 1970 s. There was a long period of inactivity, but more recent developments in NMR and mass spectral analysis and definition of the *M. tuberculosis* and *M. leprae* genomes,

and those of other mycobacteria, have resulted in a thorough understanding, not only of the structure of the mycobacterial cell wall and its lipids but also the basic genetics, biosynthesis, and roles in disease processes. Our understanding nowadays of cell wall architecture amounts to a massive "core" comprised of peptidoglycan covalently attached via a linker unit (L-Rha-D-GlcNAc-P) to a linear galactofuran, in turn attached to several strands of a highly branched arabinofuran, in turn attached to mycolic acids. The precise chemical structures of all of these entities and their linkages to one another are known. However, their spatial arrangements on the surface of mycobacteria are still a matter of conjecture. The mycolic acids are oriented perpendicular to the plane of the membrane and provide a truly special lipid barrier responsible for many of the physiological and disease-inducing aspects of *M. tuberculosis*. Intercalated within this lipid environment are the lipids that have intrigued researchers for over five decades: the phthiocerol dimycocerosate; cord factor/dimycolyltrehalose; the sulfolipids; the phosphatidylinositol mannosides; etc. The availability of relatively large quantities of the leprosy bacillus, *M. leprae*, has allowed a parallel approach. Interestingly, despite a defective genome lacking about 40% of the expected coding capacity, most of the structural features of the *M. tuberculosis* cell wall are retained. The major exception is the presence of the distinctive Phenolic Glycolipid I which had proved to be very useful in the serodiagnosis of leprosy. Knowledge of the roles of many of the lipids associated with mycobacterial cell walls in "signaling" events, in pathogenesis, and in the immune response is now emerging, sometimes piecemeal and sometimes in an organized fashion. Some of the more intriguing observations are those demonstrating that mycolic acids are recognized by CD1-restricted T-cells, that antigen 85, one of the most powerful protective antigens of *M. tuberculosis*, is a mycolyltransferase, and that lipoarabinomannan, when "capped" with short mannose oligosaccharides, is involved in phagocytosis of *M. tuberculosis*. Definition of the genome of *M. tuberculosis* has greatly aided efforts to define the biosynthetic pathways for all of these exotic molecules: the mycolic acids, the mycocerosates, phthiocerol, LAM, and the polyprenyl phosphates. For example, we know that synthesis of the entire core is initiated on a decaprenyl-P with synthesis of the linker unit, and then there is concomitant extension of the galactan and arabinan chains while this intermediate is transported through the cytoplasmic membrane. The final steps in these events, the attachment of mycolic acids and ligation to peptidoglycan, await definition and will prove to be excellent targets for a new generation of anti-tuberculosis drugs.

Program/Abstract #227

Studies on eukaryotic sialic acid O-acetylation

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Phosphorylation, methylation and acetylation are frequent chemical tools for the regulation of cell biological events. Complex carbohydrates, especially their sialic acid (Sia) moieties, are often esterified with acetic acid altering their biological and pathophysiological functions (1). Sia *O*-acetylation retards the catabolism of Sia due to the inhibition of, for example, sialidase activity and it modifies the ligand functions of these monosaccharides. Examples are some siglecs where *O*-acetylation was found to inhibit recognition of Sia. In the case of viruses esterification can also switch off recognition or, in contrast, creates new ligands for specific pathogen binding. Apoptosis is also hindered by *O*-acetylation of Sia. Various enzymes are involved in *O*-acetylation at C-4 of the pyranose ring and the side-chain of Sia, respectively. In the latter case, the primary insertion site was found to be at C-7, from where isomerization to the other positions of the side-chain may occur. We studied the sialate 7-*O*-acetyltransferase in the starfish *Asterias rubens*, bovine liver and submandibular gland, and in normal and malignant human lymphocytes using an assay with radioactive AcCoA (2). The sialate 4-*O*-acetyltransferase was studied in guinea pig liver and equine submandibular gland. CMP-Neu5Ac or the ganglioside GD3 are the best substrates for the Golgi-bound *O*-acetyltransferases.

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Program/Abstract #228 (LB-1)

Effect of N-glycans on structural stability of arylphorin

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Although N-glycosylation has been known to increase the stability of glycoproteins, it is difficult to assess the structural importance of glycans in the stabilization of glycoproteins. APA (*Antheraea pernyi* arylphorin) is an insect hexamerin that has two N-glycosylations at Asn^{196} and Asn^{344} respectively. The glycosylation of Asn^{344} is critical for the folding process; however, glycosylation of Asn^{196} is not. Interestingly, the

N196-glycan (glycosylation of Asn^{196}) remains in an immature form ($Glc_1Man_9GlcNAc_2$). The mutation of Asn^{196} to glutamine does not change the ecdysone-binding activity relative to that of the wild-type. In the present study,we determined the crystal structure of APA, and all sugar moieties of the N196-glycan were clearly observed in the electron-density map. Although the sugar moieties of the glycan generally have high structural flexibility, most sugar moieties of the N196-glycan were well organized in the deep cleft of the subunit interface and mediated many inter- and intra-subunit hydrogen bonds. Analytical ultracentrifugation and GdmCl (guanidinium chloride) unfolding experiments revealed that the presence of the N196-glycan was important for stabilizing the hexameric state and overall stability of APA respectively. Our results could provide a structural basis for studying not only other glycoproteins that carry an immature N-glycan, but also the structural role of N-glycans that are located in the deep cleft of a protein.

Program/Abstract #229 (LB-2)

Glycoengineering of human growth hormone

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Based on the structural models available, a number of N-glycosylation motifs were introduced to human Growth Hormone (hGH), a protein with no naturally occurring glycosylation motifs. Individual constructs were transiently expressed in HEK 293-6E cells and medium supernatants revealed that 7 out of 15 introduced motifs were utilized for glycosylation in different degrees. Four highly glycosylated variants remained fully active compared to the wild-type protein. By combining the highly utilized positions, a protein variant with multiple glycosylation sites was engineered and expressed from a stable CHO cell line. The resulting protein product was purified in a one-step process using anion-exchange chromatography. This made it possible to differentiate between the sialylation degree of the different fractions, with highly sialylated and more negatively charged glycoprotein eluting in the later fractions. Three different pools were characterized with regards to their glycosylation profiles. The three variants remained fully active *in vitro* and showed up to a 30-fold increase in half-life in pharmacokinetic studies in Sprague-Dawley rats. The different pools did not, regardless of differences in glycan-profiles, show significant variation in the prolonged PK-profiles.

Previous reports show that glycosylation of therapeutic proteins is a reasonable way to improve their pharmacokinetic capabilities. Here hGH, non-glycosylated protein of high commercial interest, is engineered to present multiple glycans on the surface resulting in a significant improvement of the circulatory half-life.

Program/Abstract #230 (LB-3)

Glycosylation pathways of human prostate cancer cells

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Altered glycosylation is a universal feature of cancer cells and could potentially facilitate cancer cells to escape from the immune response, and increase the potential of metastasis. The expression and activity of glycosyltransferases play critical roles in affecting the glycan structures that are found in the normal and cancerous prostate. We focus on the glycosyltransferase activities in a variety of human prostate cancer cell lines with the goal of understanding their roles in cancer cell death. Cancer cells commonly express truncated sialylated O-glycans, as well as highly branched N-glycans that can carry a high number of sialic acid residues. Studying different prostate cancer cell lines, we show high activities in polypeptide-GalNAc-transferase and Core 1 b3-Gal-transferase (C1GalT) while core 2 b6-GlcNAc-transferase (C2GnT), Core 3 b3-GlcNAc-transferase (C3GnT) and Core 4 b6-GlcNAc-transferase (C4GnT) activities are not detectable. These activities lead to the formation of truncated O-glycans. Although the expression of C2GnT was believed to facilitate the progression of many cancer types, lack of C2GnT activity in prostate cancer cells may promote survival and immune evasion through resistance to galectin-1 induced apoptosis. Furthermore, prostate cancer cells contain the activities involved in the extension and branching of N-glycans such as GlcNAc-transferase V and b4Gal-transferase. A substrate analog inhibitor of Gal-transferase was shown to block b4Gal-transferase activity in prostate cancer cells. The inhibitor was found to be specific for Gal-transferases and thus has the potential to block all extensions of N- and O-linked glycan chains of glycoproteins which would prevent the synthesis of terminal epitopes involved in cell adhesion and apoptosis.

This work was supported by the Prostate Cancer Fight Foundation.

Program/Abstract #231 (LB-4)

Plasma protein glycation association in diabetic rats

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In order to understand the complications associated with diabetes, it is important to know which structures change and the direction of changes. Diabetes is a silent disease characterized by hyperglycaemia leading to metabolic changes in lipid and in protein. Diabetes mellitus is one such types that failure to control glucose in the blood. The level of protein glycation is one of the factors under consideration in diabetic case study.

The present studies with streptozotocin induced rats have examined the correlation between the level of plasma protein glycation and the liver enzymes. The level of glucose and the creatinine are also taken into consideration for correlation study along with SGOT, SGPT, acid and alkaline phosphatases. Blood samples for both diabetic and the non-diabetic (control) rats were processed for levels of plasma proteins glycation along with other parameters for individual correlation. The result shows that there is a negative correlation between the levels of proteins glycation and the plasma glucose for both diabetic and non-diabetic rats, so far as the glucose parameter is concerned. Same results are applicable to creatinine. Liver enzymes, however, particularly SGOT have a positive correlation when compared with the level of plasma proteins glycation. A negatively correlated approach is noticed with the other liver enzymes studied. Both the phosphatases enzymes show an opposite behavior in correlation studies. The results are discussed with the changing condition of the subject.

Program/Abstract #232 (LB-5)

Glycosylated peptide random bead libraries for biomarker discovery

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There is considerable interest in discovery of O-glycoprotein epitopes (O-PTMs) recognized by natural antibodies in cancer and other patients, since molecules bearing aberrant O-PTM epitopes could be useful as diagnostic and therapeutic targets. A novel high throughput (HTP) methodology, that targets circulating autoantibodies rather than the molecules they recognize, has been developed, incorporating a random combinatorial O-glycopeptide library synthesized in the solid phase on polymer beads. The glycopeptides are attached to the beads with a cleavable linker easily released under mild conditions. Thus, beads showing positive reactivity with patient antisera in an immunofluorescence assay can be selected, the linked O-glycopeptides released, and the sequence of the reactive epitopes determined by mass spectrometric methods. In this case, rapid and sensitive sequencing of released glycopeptides incorporating one or more O-GalNAc residues has been achieved with an LTQ-Orbitrap mass spectrometer (ThermoFisher), configured for both higher-energy collision dissociation (HCD) and electron transfer dissociation (ETD), and equipped with a TriVersa NanoMate electrospray ionization (ESI)-Chip interface (Advion BioSciences). Where only a single glycosylation site is present, HCD provides sufficient sequencing data; where multiple potential glycosylation sites are possible, ETD is employed to determine the actual location(s) of O-GalNAc residues in the reactive sequence. In one example, sequencing was achieved quickly and sensitively for a peptide released from a single bead reactive with an anti-MUC1 monoclonal antibody. All components of the methodology are amenable to automated and/or HTP protocols, and the library has the potential to incorporate a broad range of structures.

Program/Abstract #233 (LB-6)

Synthesis and applications of artificial siderophores with sucrose backbone

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During the last decades, a growing interest in the development of synthetic analogues of siderophores have emerged, being useful clinical iron chelators for the treatment of iron overload diseases and for iron transport-mediated antibiotics or antimicrobial compounds. The first reports in the use of a monosaccharide backbone for artificial siderophores appeared in 2001 and 2003. Herein we report the synthesis of sucrose-based artificial siderophores. Being a disaccharide, sucrose offers different possibilities for the introduction of ligands. Its three dimensional structure also provides an optimal binding cavity for the formation of iron (III) octahedral complexes. These compounds can have applications in medical and biomedical areas.

Program/Abstract #234 (LB-7)

Dendritic cells previously exposed to mannan-binding lectin (MBL) enhance cytokine production in allogeneic mononuclear cell cultures

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Background. Mannan (or mannose)-binding lectin (MBL) recognises non-self on microbial cells and is believed to protect against various infections. Consequently, a recombinant form of human MBL has been developed for therapeutic use and is currently undergoing clinical trials. MBL can also bind to monocytes and dendritic cells but the significance of such interactions is unknown. *Objective.* We hypothesised that the presence of MBL might prevent the differentiation of monocytes into monocyte-derived dendritic cells or interfere with the development of dendritic cells in some way. *Methods*. We investigated the influence of MBL on surface antigen expression and on secretion of selected cytokines. *Results*. By these means, no influence of recombinant human MBL on dendritic cell differentiation or maturation was detected. However, mature dendritic cells pre-cultured with MBL and subsequently co-cultured with allogeneic mononuclear cells markedly promoted production of IL-1β, IL-6 and TNFα *in vitro*. *Conclusions.* This observation provides evidence that MBL can influence cellular immunity in addition to its established role as an opsonin.

Program/Abstract #235 (LB-8)

A convenient method for synthesis of glyco-nanoparticles/beads for glycan function analysis

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Carbohydrate functionalized nanoparticles, *i.e*., the *glyconanoparticles*, have wide application ranging from studies of carbohydrate-protein interactions, *in vivo* cell imaging, biolabeling, etc. We report a simple and versatile method for preparing glyconanoparticles and microbeads. This method is based on the utilization of Clean and convenient microwave irradiation energy for one-step, site-specific conjugation of unmodified carbohydrates onto hydrazide-functionalized nanoparticle/beads surface. A gold nanoparticle-based colorimetric assay that utilizes the ensemble of Concanavalin A (ConA) and gold glyconanoparticle was also presented. This feasible assay system was developed to analyze multivalent interactions and to determine the dissociation constant (Kd) for carbohydrate derivatives coated with mannose binding lectins. Shifts in UV-Vis absorption wavelength as a function of ConA-carbohydrate derivatives molar ratios were plotted and the dissociation constants were determined based on non-linear curve fitting. The dissociation constant for mannose-coated gold nanoparticles was determined at 4.4nM, while the maltose-coated gold nanoparticles suppress the binding by increasing the dissociation constant. Fluorescent glyconanoparticles and magnetic glyconanoparticles are also synthesized for glycan extraction and function analysis.

Program/Abstract #236 (LB-9)

Notch xylosyltransferases

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It has been well established that modification of epidermal growth factor (EGF) like repeats of Notch with *O*-fucose is necessary for the correct folding and expression of the receptor. The extension of O-fucose with N-acetylglucosamine by Fringe results in altered signalling by the Notch ligands Jagged and Delta. In contrast, only little is known about how *O*-glucose linked glycans are biosynthesised and influence Notch signaling. O-glucose is usually extended by two xylose residues forming the structure Xyl-Xyl-Glc-O-Ser. Whereas the O-glucosyltransferase has recently been identified via the *Drosophila* mutant rumi, both genes encoding the xylosyltransferases have not been identified, hampering studies towards the biological relevance of xylosylated O-glycans for the function of Notch and other EGF repeats containing proteins.

We have now identified two human genes encoding enzymes transferring xylose from UDP-xylose on glucose terminated acceptor in alpha 1,3 linkage. In addition we could show by mass spectrometry that the enzymes could convert Notch EGF domains that were produced in a UDP-xylose deficient cell line and carried only Glc-O to protein with Xyl-Glc-O glycans. The genes, now named GXYLT1 and GXYLT2 (glycoside xylosyltransferase) are therefore most likely encoding xylosyltransferases transferring the first xylose residue on O-glucose of Notch and probably other O-glucosylated EGF repeat containing proteins.

Program/Abstract #237 (LB-10)

A Bivalve D-galactose-binding lectin transduced the growth static signal for Burkitt's lymphoma cells mediated to Gb3 glycosphingolipid

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A 17 kDa D-galactose-binding lectin (MGL) was purified from the mussel, *M. galloprovinciaris* using melibiosyl-agarose column affinity chromatography. The amino acid sequence of MGL of 149 amino acids has been determined by Edman degradation and mass spectrometry analysis of the cleaved peptides with the aminopeptidases and CNBr. The primary structure having triple tandem repeating domain consisted of 50 amino acids was quite different from other known primary structure of animal lectins. The glycan binding of the lectin was profiled using pyridyl-aminated oligosaccharides by frontal affinity chromatography technology (FACT), appearing that MGL specifically recognized globotriose (Gala1-4Galb1-4Glc) which is the component of Gb3 glycosphingolipid. MGL dose-dependently inhibited the growth of the Burkitt's lymphoma Raji cells which expressed Gb3 glycosphingolipid on the cell surface, however it did not inhibit the cell proliferation of control K567 cells. Both the binding of anti-annexin V antibody on the cell surface and incorporation of propidium iodide to nuclei after treatment of the lectin have also detected by fluorescence-activated cell sorter (FACS). The cell proliferating inhibition of Raji cells by MGL was totally cancelled by the co-presence of D-galactose in the assay, indicating that MGL transduced the Gb3-dependent cell static signal against Burkitt's lymphoma cells.

Program/Abstract #238 (LB-11)

Frontal affinity chromatography for glycan binding profiling of galectin-1 purified from American Bullfrog (Rana catesbeiana) oocyte

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A 15 kDa b-galactoside-binding lectin (Galectin-1) was purified from the oocytes of the American bullfrog, *Rana catesbeiana* by lactosyl-agarose column chromatography. The glycan binding profile was studied using 61 pyridyl-aminated oligosaccharides by frontal affinity chromatography technology (FACT). Glycolipid-type glycans, human blood type-A-hexasaccharide (GalNAca1-3(Fuca1-2) Galb1-4GlcNAcb1-4Galb1-4Glc) was found to exhibit the strongest ligand binding to the galectin while Forssman antigen (GalNAca1-3GalNAcb1-3Gala1-4Galb1-4Glc) and also type-A-tetrasaccharide (GalNAca1-3(Fuca1-2)Galb1-4GlcNAcb1-4Glc) were extensively recognized. On the other hand, type-B tetraose (Gala1-3(Fuca1-2)Galb1-4Glc) and type H tetraose (Fuca1-2Galb1-4Glc) were significantly less recognized by the galectin-1 compared to type-A tetraose. The kinetics of affinity of galectin-1 to type-A oligosaccharide was analysed by surface plasmon resonance using neoglycoprotein with type-A oligosaccharides. The k_a and k_d values were determined to be 4.74×10^4 $M^{-1}s^{-1}$ and 3.12×10^{-2} s^{-1}, respectively. *Rana catesbeiana* oocyte galectin adhered to human rhabdomyosarcoma cells dose dependently and the activity was specifically cancelled by the neoglycoprotein. The primary structure of peptides had similarity with galectin family which was determined by automatic Edman degradation using protein sequencer. Therefore, galectin-1 from *R. catesbeiana* oocytes possesses different and rare glycan-binding properties from typical members in galectin family. Finally, FACT will give us a new clew to impose the endogenous glyco-ligands of the lectin in the animal to understand the important role of the lectin for biological phenomena.

Program/Abstract #239 (LB-12)

NMR spectroscopy to elucidate the interaction between the transmembrane receptor CCR5 and glycopeptides from the GP120 of HIV

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Saturation transfer difference NMR spectroscopy (STD NMR) is very useful to elucidate protein-ligand interactions. Saturation of a protein is transferred to a ligand if there is an exchange between the bound and the free ligand. The difference of a spectrum with protein saturation and a spectrum without represents a spectrum that shows only molecules that bind to the receptor.

The technology is very robust and can be applied to proteins with a molecular weight larger than 10kD. In fact, there is no upper limit to the size of the protein. The protein can be anchored into the membrane of liposomes or native cell walls. STD-NMR is very sensitive if the off-rate is fast. We have been working with as little as 30 pmol (a few micrograms) of protein. As a result, for the ligands binding constants and binding epitope can be obtained.

The use of STD NMR for assaying the interactions between ligands and cellular transmembrane receptors is described. STD NMR can be used to analyze the binding of small molecules to large membrane integrated receptors in living cells using a newly developed variant of the STD protocol called STDD (Saturation transfer double difference) spectroscopy.

In order for HIV to infect human cells, two human receptor proteins, CD4 and CCR5, have to interact with a highly glycosylated viral protein, the gp120.We analyzed, the interaction of a glycopeptide derived from the hypervariabel loop V3 of gp120 with the human seven helix transmembrane chemokine receptor, CCR5, is described. The receptor CCR5 is a G-protein coupled receptor (GPCR). This interaction is crucial for the HIV to infect human macrophages, which occurs during the asymptomatic early phase of HIV infections. STDD NMR and

Biacore surface plasmon resonance experiments allow the characterization of the binding epitope of the gp120 derived glycopeptide in its interaction with the CCR5 receptor.

Program/Abstract #240 (LB-13)

Human intestinal starch digestion enzymes maltase-glucoamylase and sucrase-isomaltase

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Two human intestinal enzymes are responsible for the post-amylase processing of amylose and amylopectin derived from nutritional starch. These two enzymes, maltase-glucoamylase and sucrase-isomaltase, each consists of two GH31 glycoside hydrolase enzymatic domains. Biochemical studies suggest that these four domains possess somewhat different substrate specificity and enzyme kinetic properties, and it is proposed that they act cooperatively to produce glucose under different physiological conditions.

We have undertaken structural analysis of these four related enzyme domains in order to rationalize the characteristics of their structures that give rise to their different properties. One motivation is to develop small-molecule inhibitors that can control individually each of the four activities, as critical reagents for investigating their complementary roles in nutrition. These compounds would form the basis for interventions for nutritional disorders, including Type II Diabetes and obesity.

Our progress to date features the crystallographic structures of the initial (N-terminal) GH31 domains of both maltase-glucoamylase and sucrase-isomaltase. Structures in the presence of inhibitors have permitted the mapping of the substrate binding regions and proposals concerning the structural basis for different substrate specificities. We have also expressed both the C-terminal domains in insect cells by baculovirus infection, and purification of those is underway.

Program/Abstract #241 (LB-14)

The Leu125Val and Ser563Asn gene polymorphisms combination of platelet endothelial cell adhesion Molecule-1(PECAM-1) increases endothelial function

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We have explored the *in vitro* effects of two recently reported single nucleotide polymorphism (SNPs) of PECAM-1 gene, V125L and Asn563Ser, shown to be associated with atherogenesis in coronary artery disease patients. A PECAM-1-nil endothelial-like cell line, Ren cells, was stably transfected with expression vector pIRES Neo2 [Ren (-)], PECAM-1 cDNA constructs over expressing wild type (125 Leu and 563 Ser), PECAM-1 cNDA constructs [Ren (+)/WT] and cNDA constructs containing the combination of 125Val and 563Asn gene polymorphisms [Ren (+)/PM]. PECAM-1 gene and protein expressions were measured by real time PCR and Western blot assays. The effects on trans-endothelial migration (TEM) of monocytes was also examined. The level of PECAM-1 protein in Ren (+/PM) lysate (1.4 folds) and cytosol fractions were increased 1.4- 4-fold compared to Ren (+/WT) cells. Moreover, the secretion of soluble PECAM-1 was also increased (1.6 folds) in the spent medium. In conjunction, the aggregation and TEM were increased ~2.5-fold and 1.8fold in Ren (+/PM) cells compared with Ren (+/WT) cells.

In sum, our observations point to a patho-physiological role of PECAM-1 gene polymorphisms contributing to increased endothelial function, atherosclerosis and thrombosis.

Program/Abstract #242 (LB-15)

A potent glycomimetic selectin antagonist (GMI-1070) in clinical trials for sickle cell disease

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The selectins play major roles in the onset and severity of vaso-occlusive crisis (VOC) in patients with sickle cell disease. During crisis, leukocytes adhere to the blood vessel walls and form aggregates with sickle rbc's thereby slowing and blocking blood flow resulting in painful injury to vital organs and increased morbidity and mortality. GMI-1070 is a small molecule glycomimetic that was rationally designed based on the bioactive conformation of sLe^x bound to E-selectin. Over 50% of neutrophils are inhibited from binding to immobilized E-selectin under flow at 1ug/ml demonstrating an IC_{50} in the nanomolar range. Phase 1 results from 72 healthy individuals show GMI-1070 has a serum half life of 7 to 8 h. There were no serious adverse events and over 90% of the drug is excreted intact in the urine thereby eliminating metabolic concerns.

In a Phase I/II study on sickle cell patients not in crisis (steady state), GMI-1070 also had a similar serum half life. A dosing regimen with GMI-1070 maintained blood levels over 20ug/ml for 24 h. At these concentrations, GMI-1070 demonstrated complete efficacy in inhibiting neutrophil adhesion *in vitro* and in normalizing blood flow and eliminating sickle rbc/neutrophil adhesions in a sickle cell mouse model of VOC. Steady state sickle cell patients have elevated activated neutrophils. The levels of E-selectin in the blood of sickle cell patients also correlates with mortality. As E-selectin is known to activate neutrophils, treatment of steady state sickle cell patients with GMI-1070 in a Phase I/II clinical trial reduces adhesion of their neutrophils to immobilized E-selectin and ICAM-1 and also inhibits their constitutive activation of neutrophils as determined by the reduction of activated integrin expression.

Program/Abstract #243 (LB-16)

Impact of N-glycosylation on glucagon receptor function

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Asn (N)-linked protein glycosylation is widely found in all domains of life, and is the most abundant modification of secretory and transmembrane proteins. The complex-type N-glycan structures of proteins at the cell surface are ligands for galectins. The galectin-glycoprotein interaction forms a dynamic lattice that regulates interaction of cell surface cytokine receptors and nutrient transporters with other microdomains including coated-pits and caveolae. Mice deficient in Mgat5, the major β1,6-N-acetylglucosaminyltransferase, lack tri- and tetra-antennary N-glycans, resulting in an altered distribution of glycoproteins at the cell surface due to a reduction in affinity for galectins. Mgat5-/- mice are resistant to weight gain on an enriched diet, hypersensitive to fasting and mildly hypoglycaemic. Here we show that Mgat5-/- mice have increased insulin sensitivity, delayed gluconeogenesis and are less sensitive to glucagon injections. This phenotype points to impaired glucagon receptor function. In fact, localization studies with primary hepatocytes showed that the glucagon receptor in Mgat5-/- cells is located in intracellular vesicles in contrast to a cell surface localization of wild type cells. Furthermore, we demonstrated that Mgat5-/- hepatocytes are also less sensitive to glucagon stimulation. Endocytosis experiments in transiently transfected CHO wild type and Lec mutant cells confirm that lack of N-glycosylation leads to an increased receptor internalization. Finally, the impact of sugar-nucleotide metabolism on glucagon receptor function was investigated in Mgat5-/- mice by supplementation with GlcNAc. The resulting changes in glucose homeostasis indicate that glucagon receptor function can be enhanced by the metabolite supply to hexosamine pathway and N-glycan branching.

Program/Abstract #244 (LB-17)

Calculating theoretical binding energies for hemagglutinin-host cell receptor interactions—computational prediction of hemagglutinin specificity

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Influenza hemagglutinin (HA) is a type I integral membrane glycoprotein responsible for binding terminal sialic acid host cell receptors to facilitate viral entry by endocytosis. The HAs of avian influenza exhibit specificity for terminal sialic acid residues a(2–3) linked to galactose. This sequence is found predominantly on the intestinal epithelium of birds. In contrast, the HAs of human-infective influenza exhibit specificity for receptors containing a(2–6) linkages, the main type found on the epithelial cells of the human upper respiratory tract. All strains of influenza A are known to have originated in avian species and crossed over to humans and other mammals via mutation of their individual gene segments. Therefore, a key step in interspecies transmission is mutation of an avian HA to alter its binding preference from a(2–3) to a(2–6) host cell receptors. As few as one or two amino acid mutations may be sufficient to alter avian HA specificity and allow infection in humans.

We report theoretical binding energy data for human and avian HA-receptor complexes based on crystallographic structures of the 1934 human H1, generated via computational simulation using the GLYCAM/AMBER force field. The data demonstrate the ability of theoretical methods to accurately quantitate the effects of amino acid mutations on the binding energy of HA-receptor interactions. For mutations known to alter specificity, the data indicate an appropriate change in HA binding preference. For mutations known to attenuate binding, the data indicate a significant decrease in binding affinity. The results of this study provide structural and quantitative insight into the origin of the affinities of HA-receptor binding interactions. In addition, these studies provide validation of current

theoretical methodologies, which could pave the way for computational protocols to predict HA specificity given amino acid sequence.

Program/Abstract #245 (LB-18)

Developing insights into the *O*-GlcNAc transferase substrate peptide; sequon identity from dissecting the results of a peptide library screen

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The transient *O*-GlcNAc modification of nucleocytoplasmic proteins is carried out by UDP-GlcNAc:polypeptidyl transferase (OGT) that modifies selected serine and threonine residues. *O*-GlcNAcylation has been reported in many cellular processes including transcription, the stress response, glucose homeostasis, apoptosis and signal transduction but little is known about the identity of the consensus sequence or the "sequon" OGT recognises.

To gain insight into the existence of a consensus sequence, *Hs*OGT was screened in 384 format, in triplicate, against a library of 760 biotinylated peptides using a scintillation proximity assay. Tritiated UDP-GlcNAc was used as a tracer and a mutant form of α-A crystalline (NH_2 -AIPVSRA***E***K(biotin)-COOH, Leavy & Bertozzi 2007) was used as a control peptide. *O*-GlcNAcylated peptide was captured by streptavidin coated FlashPlates and the emitted light quantified on a scintillation counter. The resulting heat maps identified several peptides of interest which were re-synthesised to confirm the results of the screening campaign. Peptides of interest were then dissected experimentally by electron thermal dissociation (ETD) and alanine scanning of key residues to elude the sites of *O*-GlcNAcylation and the necessity of the flanking residues.

Program/Abstract #246 (LB-19)

The GT2 enzyme NodC as a model for studying processive chitin synthase

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The Glycosyl Transferase family 2 (GT2) contains enzymes that are involved in the processive synthesis of polysaccharides such as hyaluronan, cellulose and chitin. To date no structural information is available for any of these enzymes and therefore the details of their processive catalytic mechanism remains unknown. We are trying to understand the catalytic mechanism of chitin synthase by studying NodC, a GT-2 chito-oligosaccharide synthase from a Rhizobium species. NodC synthesizes the backbone of the rhizobial nodulation factor, consisting of five modified beta(1,4)-linked N-acetyl-glucosamine residues. Here we present the preliminary results of expression studies of full-length NodC integrated into the bacterial membrane. We have developed a novel, non-radioactive, coupled assay to detect enzymatic activity. This has, for the first time, allowed determination of kinetic parameters and metal dependency of NodC. Futher more we show with this assay that several well-known chitin synthase inhibitors also potently inhibit NodC. A series of amino acids conserved between NodC and the chitin synthases were targeted by mutagenesis, revealing residues involved in catalysis and further showing that NodC is a useful model for eukaryotic chitin synthase.

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